NUTRITIONAL ECOLOGY OF OLD-WORLD FRUIT BATS: A TEST OF THE CALCIUM-CONSTRAINT HYPOTHESIS

By

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To Darrin

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Chair: Dr. Stephen R. Humphrey
Co-Chair: Dr. Tom Kunz
Department: Wildlife Ecology and Conservation

Although most studies argue that energy or protein is the most limiting component of the fruit bat diet, the calcium-constraint hypothesis proposes that reproduction in bats may be constrained by calcium rather than energy.

To test this hypothesis, I experimentally tested if bats attempted to increase their calcium ingestion through preferential selection and consumption of calcium-rich foods. This research used four different species of fruit bats in three different geographic locations to test this theory. The various methods used included mineral metabolism trials, fruit choice and mineral block experiments, nectar concentration trials, and studies of habitat use by radiotelemetry.

Results indicated that fruit bats seemed to base their food choices on the sugar content of fruits rather than the calcium content. Fruit bats preferred high-sugar agricultural fruits in all experiments, but bats did not meet their mineral requirements by

consuming them. To compensate for mineral deficiencies resulting from reproduction or rapid growth, fruit bats may demonstrate an additional preference for concentrated mineral sources. Reproductive females and subadult bats appeared to select for additional calcium by consuming leaves and by using the calcium blocks. Future work should examine larger numbers of reproductive females and should observe bat foraging throughout the entire night to look for temporal deviations in resource use. Population persistence of Tongan fruit bats through time will reveal if these dietary choices are adaptive or maladaptive.

Fruit bats act as seed dispersers and pollinators and are considered keystone species on isolated oceanic islands. Hunting, hurricanes, and habitat loss threaten bat populations. Native forest roosts are an essential resource component of the landscape for bat populations. This study suggests envisioning a nutrient landscape and then evaluating its components as they contribute to the needs of the bat population. A full explication will provide for conservation planning.

CHAPTER 1
INTRODUCTION

Old-World fruit bats, or flying foxes, opportunistically feed on a wide variety of plants. Their diet includes fruits, flowers, leaves, shoots, buds, nectar, and pollen of tropical forest trees and shrubs (Start and Marshall 1974, Marshall 1985, Pierson and Rainey 1992, Wiles and Fujita 1992, Kunz and Diaz 1995, Banack 1996, Bonaccorso 1998, Tan et al. 1998). Current estimates are that flying foxes consume the fruit of 136 genera, flowers of 97 genera, and leaves of 10 genera (Courts 1998). Food choice may be influenced by a myriad of factors, including energy needs, requirements for specific nutrients, reproductive status, constraints of the digestive system, abundance, diversity, and seasonality of different food items, and competition and predation (Fleming 1988, Oftedal 1991).

Fruit bats in the wild appear to meet their nutrient needs by consuming large quantities of a wide variety of native fruits (Dempsey 1999). Their food tends to be conspicuous, abundant, and easily harvested within clumps (Mickleburgh et al. 1992). Hundreds of bats may descend on a locally and temporarily abundant food source until it is depleted (Pierson and Rainey 1992, Wilson and Engbring 1992). Fruit bats are often regarded as "sequential specialists," favoring preferred resources among a group of foods as they become seasonally available (Marshall 1983, 1985, Banack 1998).

The largest genus of the Old World fruit bats, *Pteropus*, is primarily an island-dwelling taxon, with 97% having some or all of their distribution on islands (Mickleburgh et al. 1992, Pierson and Rainey 1992). Fruit bats are crucial to establishing

and maintaining forest composition on isolated oceanic islands that have a characteristically limited suite of animal pollinators and dispersers (Thornton et al. 1990, Cox et al. 1991, Elmqvist et al. 1992, Cox and Elmqvist 2000, Cook et al. 2001). Flying foxes are highly mobile and can travel 40-60 km to reach a feeding area (Marshall 1983, Rainey et al. 1995, Banack 1996). Thus, they are able to transport seeds great distances as they drop or defecate them while in flight (Rainey et al. 1995, Shilton et al. 1999). Fruit bats are particularly important to tropical forest regeneration following natural catastrophes or forest destruction by humans (Bonaccorso and Humphrey 1978, Whittacker and Jones 1994, Thornton et al. 1996,), and they can influence the composition and distribution of food resources within the landscape (Kunz 1996). Although contributions of fruit bats to tropical forests are well documented, the factors that influence food choice are still highly debated and largely unknown. Factors that influence food choice can be evaluated within the discipline of nutritional ecology.

Nutritional Ecology

Nutritional ecology includes the study of organic and mineral nutrients. Organic nutrients consist of fiber, carbohydrates (fiber and soluble carbohydrates), protein (nitrogen), fat and/or energy. Mineral nutrients are inorganic elements and include calcium, phosphorus, and iron. Minerals required in gram quantities in the body are referred to as macrominerals and consist of calcium, phosphorus, sodium, chlorine, potassium, magnesium, and sulfur. Macrominerals are important structural components of bone and other tissues and play vital roles in the maintenance of acid-base balance, osmotic pressure, and membrane electrical potential (McDowell 1992, NRC 2001). Minerals required in milligram or microgram amounts are referred to as trace minerals. This group includes copper, iodine, iron, manganese, molybdenum, selenium, zinc, and

perhaps chromium and fluorine. Trace minerals are present in very low concentrations and often serve as components of metalloenzymes, enzyme cofactors, or hormones in the endocrine system (NRC 2001). Studies of dietary minerals quantify and compare mineral concentrations in foods consumed by animals. The study of minerals, particularly calcium, represents the primary focus of my research.

Calcium

About 98% of the calcium in the body is located within the skeleton (McDowell 1992). Calcium is essential for the formation of skeletal tissues, transmission of nervous tissue impulses, excitation of skeletal and cardiac muscle contraction, blood clotting, and as an important component of milk (NRC 2001). The calcium concentration of plasma must be maintained at a relatively constant value of 1 to 1.25 mM to ensure normal nerve membrane and muscle end plate electric potential and conductivity (NRC 2001). Vertebrates have evolved an elaborate system to maintain calcium homeostasis whenever there is a loss of calcium (NRC 2001). The parathyroid glands monitor the concentration of calcium in carotid arterial blood. When levels drop, calcium can be replaced by resorption of calcium stored in bone, by increased calcium absorption, or by reducing urinary calcium loss (NRC 2001). Active transport of calcium, an energy-requiring process, appears to be the major route of calcium absorption and is controlled by the hormone form of vitamin D (Wasserman 1981, Bronner 1987, Holick 2002a, 2002b). If dietary calcium is severely deficient for a prolonged period, an animal can develop severe osteoporosis to the point of developing fractures (Radostits et al. 1994). Despite the skeletal changes that result from prolonged calcium deficiency, plasma calcium levels are homeostatically maintained and will only be slightly lower than normal (NRC 2001).

Previous Work in Bat Nutrition

Previous nutritional studies of fruit bats identified the organic nutrient protein (nitrogen) as the major limiting nutrient in the diet (Thomas 1984, Herbst 1986, Steller 1986). It was previously thought that fruit bats consumed only fruits and did not supplement their diet with other foods (Thomas 1984, Herbst 1986).Yet fruits were considered nutrient-poor because of their low fat and protein content (Mattson 1980, Herrera 1987, Witmer 1998). Daily fruit intake and preference were thought to be dictated by protein rather than energy content of the diet. It was thought that bats over-consumed energy to obtain adequate protein (Thomas 1984)

Later research showed that the diet of fruit bats includes much more than just fruit (Banack 1996, Courts 1998). Fruit bats have been reported as deliberately ingesting insects, pollen, and leaves, possibly to provide extra protein in the diet (Drew 1988, Mickleburgh et al. 1992, Kunz and Diaz 1994, Kunz and Ingalls 1995, Kunz 1996, Courts 1998). Thus, protein may not be a limiting nutrient in the bat diet. Instead, minerals such as calcium may be deficient in the diet of bats (Barclay 1995). Barclay (1994, 1995) proposed that because of bat's adaptation to flight, reproduction of females bats is most constrained by their intake of calcium rather than their intake of protein or energy.

Bats and Flight

The unique adaptation of flight in bats imposes certain restrictions on their reproduction and development not experienced by other mammals. The wing skeleton of a growing bat must acquire structural and material characteristics that will enable it to withstand the mechanical pressures of flapping flight (Bernard and Davison 1996). This includes adequate mineralization to confer strength and stiffness and to resist torsional

and/or bending stresses (Papadimitriou et al. 1996). Bats and similarly sized terrestrial mammals produce litters with a mass averaging 25% that of the female (Kurta and Kunz 1987, Hayssen and Kunz 1996). Compared to other mammals, bats raise their young to a significantly larger size because young cannot fly and gain independence until they are almost fully grown (Barclay 1995). Juvenile bats are unable to fly or forage independently until they have achieved approximately 70% of adult mass and more than 95% of adult skeletal size (Kurta and Kunz 1987, Barclay 1995,).

Rodents typically produce small litters that are weaned quickly at 30-44% of adult size and obtain some of their nutrition by foraging for themselves (Millar 1977, Barclay 1995). In contrast, maternal milk is the only energy and nutrient source for dependent young bats. Juvenile flying foxes associate with their mothers for up to a year (Kurta and Kunz 1987, Mickleburgh et al. 1992, Pierson and Rainey 1992) and will opportunistically nurse if in close proximity, especially in captivity (D. LeBlanc, pers. comm.). Thus, near the end of lactation, females provide total nutrition to bat offspring that are nearly of adult size (Kunz et al.1995, Kunz and Stern 1995, Hood et al. 2001). Overall, bat pups are more expensive, in terms of energy and nutrients, to a female bat than is each young to an equivalently sized terrestrial mammal (Barclay 1995). Each young requires a large parental investment which may restrict the total number of young that can be raised (Barclay 1994, Kunz and Hood 2000).

Reproductive Costs

Several studies indirectly addressed the question of calcium demand during pregnancy and lactation, and showed that these are periods of calcium stress for bats (Kwiecinski et al. 1987a, 1987b, Studier et al.1991, Sevick and Studier 1992, Studier et al. 1994a, 1994b, Bernard and Davison 1996). Nutritional requirements for females

increase dramatically during reproduction, and females may be in negative calcium balance from the onset of pregnancy to the end of lactation (Bernard and Davison 1996).

Females bats bear almost the entire mineral cost of raising their offspring by allocating their own skeletal calcium reserves to build the skeletons of their young (Bernard and Davison 1996, Papadimitriou et al. 1996). The bones of females become more porous as stored calcium is depleted during prolonged lactation (Sevick and Studier 1992, Radostits et al. 1994). The excessive calcium demands of raising several young in sequential years can result in osteoporosis in female bats, particularly in the mandible and the long bones of the wings (Kwiecinski et al. 1987a). Through time, this has the potential to decrease a female's fitness. The increased risk of wing-bone fractures can impede her ability to fly and forage. Tooth loss and the subsequent inability to chew fruits and leaves could affect longevity, fitness, and overall health (Barclay 1995).

Keeler and Studier (1992) found that among reproductive female bats, all caloric requirements were met, but calcium intake was one-tenth the estimated requirement. For a lactating female, inadequate calcium can result in low milk production (McDowell 1992). Several factors can influence the postnatal growth of mammals, including age, nutritional and hormonal condition of the mother, and milk quality and quantity (Hoying and Kunz 1998, Kunz and Hood 2000). For nursing bat pups, inadequate milk results in inhibited growth and reduced mineralization of bone, which can result in lameness and bone fractures (Radostits et al. 1994, NRC 2001). Studies of other mammals showed that inadequate quantities of calcium in the diet can affect fecundity, number of litters, and survival of offspring (Batzli 1986, Delgiudice et al.1990). Calcium may be a limiting nutrient for reproductive bats that could influence population density through time.

Relieving Mineral Deficiencies

Bats may be able to delay or reduce the effects of calcium deficiency by feeding on calcium-rich foods or on concentrated calcium sources. For example, O'Brien et al. (1998) proposed that frugivores eat figs because they have higher calcium concentrations than many other native and agricultural fruits. Calcium concentration of figs in American Samoa were three times that of other native fruits, and over ten times more concentrated in calcium than agricultural fruits on the island (Nelson et al. 2000a). Leaves are also especially rich in calcium and are consumed by bats (Lowry 1989, Kunz and Ingalls 1994, Kunz and Diaz 1995,Tan et al 1998, Ruby et al. 2000). Concentrated sources of calcium can supplement the diet of fruit bats, and may be important in times of greater physiological need such as pregnancy and lactation.

Dissertation Focus

Studies of female food choices that result from the energetic and mineral demands of pregnancy and lactation are limited. Robert Barclay proposed bold new ideas on what may motivate female food choice during reproduction (Barclay 1994, 1995). My research tests Barclay's "calcium-constraint hypothesis" which proposes that reproductive females are more constrained by calcium than energy in their diet (Barclay 1994, 1995). I examined whether bats attempted to increase their consumption of deficient nutrients through dietary choice.

It is a challenge to conduct nutrition work on Old World fruit bats because the requirements for calcium and all other minerals needed to maintain bat health are currently unknown. Previous dietary work most often used mammalian standards or standards for rats to determine bat requirements, despite different diets, digestive systems and metabolic rates of bats (Oftedal and Allen 1996). Thus, one of the goals of my

research was to establish mineral requirements for flying foxes that could be used for future wild and captive nutrition studies. By offering free-choice diets to fruit bats and quantifying mineral consumption, I hoped to create a mineral profile of bat feeding.

This research focused on three different geographic regions and four different fruit bat species that represent three different size classes of bats. Each species was appropriate to answer a specific question on fruit bat feeding and nutrition. Research was performed in Papua New Guinea on the tiny fruit bats (18-20 g) *Macroglossus minimus* and *Syconycteris australis*, in American Samoa on the mid-sized fruit bat (300-600 g), *Pteropus tonganus*, and on one of the world's largest fruit bats (1000-1500 g), *Pteropus vampyrus*, at the Lubee Foundation, Inc., in Gainesville, Florida.

Most of the nutritional work in my research involved the Tongan fruit bat in American Samoa. This species was chosen because it is mid-sized (300-600 g), is one of the most widely distributed of all *Pteropus* species (Koopman and Steadman 1995, Miller and Wilson 1997), and it feeds in both native and agricultural habitats (Pierson and Rainey 1992, Banack 1996). Together, these features suggest that this species is highly adaptable and is an excellent general model. Therefore, results of nutritional studies in this species should be widely applied to many other flying fox species. The Tongan flying fox was used to generate new information on wild fruit bat mineral levels, leaf consumption, use of concentrated mineral sources, and habitat use while foraging.

One of the world's largest flying foxes, *Pteropus vampyrus*, was used to study mineral consumption in a population of captive lactating females and their dependent pups. The large size of *P. vampyrus* may result in excessive mineral requirements as females attempt to meet the nutrient demands of a large pup for up to a year. Thus,

quantifying the mineral content of the diet that can meet the requirements of this large flying fox can be helpful in formulating diets for reproductive females of other captive flying fox species. Mineral absorption values can also be compared to determine if wild Tongan fruit bats are mineral deficient.

Because Barclay (1995) argued that reproductive females were more constrained by calcium than energy, I examined energy consumption in two species of blossom bats in Papua New Guinea. These species are among the smallest members of the Old World fruit bats. They have a high metabolic rate and little or no means of storing large energy reserves (Lemke 1984), so they may be energy-sensitive foragers limited by food availability (Law 1992, 1993b, 1994a). Different nectar solutions representing different energy concentrations were given to the bats in choice trials to determine whether energy was a limiting nutrient for these species.

I used two methods new to the study of bats. To quantify bat mineral consumption, I used apparent absorption, a method commonly used in animal science but rarely in wildlife ecology because of the need to use captive animals. This method accounts for minerals in the both the food and feces, and for minerals lost in the fibrous pellet expelled while fruit bats feed (Lowry 1989, Kunz and Ingalls 1994). Also new to the study of bats is the idea of nutritional landscape ecology, which examines whether bats use mineral-rich habitats while foraging throughout the landscape.

For all these studies, I predicted that bats will seek out calcium-rich sources to supplement their diet. The additive effect of the constraints of flight, reproductive costs, and calcium-poor diets should make calcium consumption a priority.

CHAPTER 2
RESEARCH STUDY SITES AND SPECIES

Flying Foxes

The Order Chiroptera consists of two suborders: Microchiroptera and Megachiroptera. Current estimates suggest that Megachiroptera separated from Microchiroptera 50.2 million years ago (Bastian et al. 2001). Unlike Microchiroptera, which has a global distribution, members of Megachiroptera, also called Old World fruit bats, are limited to the Paleotropics. Megachiroptera comprise the single family Pteropodidae, which contains 42 genera and 191 species (N. Simmons, pers.comm.). Flying foxes are members of the genus *Pteropus*, which contains 58 species (Koopman 1993). Almost 97% of flying fox species are island dwelling, and 35 species are confined to a single island or island group (Mickleburgh et al. 1992). Members of the genus *Pteropus* range from Madagascar to India, and from Southeast Asia to Australia, and reach as far east as the Cook Islands (Pierson and Rainey 1992).

The genus *Pteropus* feeds on fruit, leaves, nectar and pollen of trees found within both native forests and agricultural areas. Across their range, *Pteropus* species are known to visit over 92 genera of plants in 50 different plant families (Marshall 1985, Wiles and Fujita 1992). Fruit bats are important pollinators and seed dispersers in tropical forest ecosystems (Fleming 1988, Rainey et al. 1995, Banack 1996). On many isolated oceanic islands with depauperate pollinator and seed disperser faunas, flying foxes are the only animals capable of carrying large-seeded fruits. In island ecosystems of the south Pacific, flying foxes are considered keystone species because their extinction could result in a

significant decline in both native forest diversity and regeneration (Cox et al. 1992, Rainey et al. 1995). Their role as long distance seed dispersers (Shilton et al. 1999) further demonstrates their critical role in maintaining forest structure and integrity. Seed dispersal by bats may increase seed survival by decreasing seed predation and increasing the chances of landing in favorable microhabitats (Jantzen et al. 1976, Augspurger 1983).

Flying foxes are phytophagus and consume fruits, pollen, flowers, nectar and leaves of plants (Marshall 1985). They process fruit by pressing the tongue against the palate to break up the fruit and ingest the pulp and juices. Flying foxes also consume leaves as a regular part of their diet (Kunz and Ingalls 1994, Kunz and Diaz 1995). They chew the leaves and swallow the juice, ejecting the fiber portion as a small compressed pellet (Lowry 1989). The ingestion of mostly the liquid portion of fruits and leaves results in a food transit time as low as 20 minutes (Tedman and Hall 1985). The advantage of this foraging strategy to a flying mammal is reduced bulk and wing loading, and reduced energy expenditure traveling to foraging areas (Kunz and Ingalls 1994).

Most flying fox species give birth to one offspring per year starting when they are two years old (Pierson and Rainey 1992). Gestation periods range from 4-6 months followed by a rearing interval of equal length, although young may stay associated with their mothers for up to a year (Banack 1996, Hall and Richards 2000). A few species (*P. mariannus yapensis* in Yap, *P. molossinus* in Pohnpei, and *P. tonganus* in Samoa) may have more than one birth peak per year (Pierson and Rainey 1992). Birth peaks are often correlated with seasonality and resource availability (Heideman 1995, Racey and Entwistle 2000). Bats of the genus *Pteropus* typically give birth to a single young, but twinning can occur.

Habitat alteration and habitat loss are the primary reasons for declining populations of flying foxes (Cheke and Dahl 1981, Fujita and Tuttle 1991, Mickleburgh et al. 1992, Pierson and Rainey 1992). Many species, particularly those inhabiting mangrove swamps and lowland forest have lost critical roosting areas (Mickleburgh et al. 1992). Fruit bat populations are also heavily influenced by human depredation and tropical storms (Wodzicki and Felton 1980, Cheke and Dahl 1981, Heaney and Heideman 1987, Craig et al. 1994a, 1994b, Loebel and Sanewski 1987).

American Samoa

The Samoan islands are a biogeographical unit politically divided into American Samoa, an unincorporated territory of the United States, and Samoa, a sovereign country. They lie in the South Pacific Ocean (14° S, 170° W), approximately 4000 km southwest of Hawaii and 3000 km northwest of New Zealand. American Samoa comprises Tutuila, Aunu'u, the Manu'a Islands (Ta'u, Olosega, and Ofu), Rose Atoll, and Swains Island. The largest island in American Samoa is Tutuila, which is 142 km^2 in area, supports 90% of the human population, and contains the capital village of Pago Pago (Craig and Syron 1992).

The islands are volcanic in origin, having risen from hot spots on the ocean floor in the late Pliocene or early Pleistocene (Kear and Wood 1959). The islands are now highly eroded, resulting in extremely steep topography and deeply cut valleys. The climate is warm and humid year-round and considered moist tropical, with an average annual temperature of 25°C. There are two distinct seasons, the wet and dry season, although rain falls 300 days/year (Amerson et al. 1982). The wettest months are October through March and rainfall averages 3200 mm annually (NOAA 1996).

Samoa lies within the South Pacific hurricane belt and is subject to hurricanes and tropical storms. The Samoan archipelago was battered by three intense storms in 1986, 1990, and 1991. These were the most severe storms to occur in Samoa in over 160 years, with each sustaining winds between 200 km/h and 240 km/h (Elmqvist et al. 1994). Because the storms occurred so close together in time, they resulted in extensive damage to native forest trees. Fruit bat populations were severely decimated due to loss of food, roosts, and increased hunting immediately after the storms (Craig et al. 1994b, Elmqvist et al. 1994, Pierson et al. 1996, Hjerpe et al. 2001). A sharp rise in air temperature over the past decade (NOAA 1999) suggests further climatic uncertainty and a probable increase in the frequency of hurricanes in the area (Craig et al. 2000).

Pacific island archipelagos display very high levels of endemism; typically 30%-50% of the plants occur nowhere else (Brautigam and Elmqvist 1990, Cox et al. 1992). More than 326 genera of vascular plants can be found in the Samoan archipelago (Christophersen 1935) and at least 68 are endemic plant species (Amerson et al. 1982). The affinities of most plants are Australian or Malesian (Whistler 1992). Paleotropical rainforest is the natural vegetation of Samoa.

Rainforest originally covered nearly the entire surface of Samoa. Mature rain forest is now restricted to the least accessible areas such as steep interior slopes and the wet, cool montane regions away from villages. Rainforest is typically a tall forest with a canopy up to 30 m in height (Whistler 1992). Rainforest trees in American Samoa produce fleshy fruits adapted for dispersal by frugivorous birds and bats (Freifeld 1998). The four types of rainforest are coastal, lowland, montane, and cloud forest, and each is most easily distinguished by the plant species found within them (Whistler 1992).

Anthropogenic disturbances such as shifting cultivation and the development of agroforestry have replaced much rainforest with secondary forest and agricultural lands. Secondary forest is less diverse than mature rainforest, and is dominated by shade-intolerant trees that quickly establish in disturbed areas (Whistler 1992 Freifeld 1998). Cultivated lands consist of local slash-and-burn plots growing together in a mix of forest and agricultural plants and are found in valleys and near villages (Cole et al. 1988).

The flora of American Samoa includes over 800 species of angiosperms that are pollinated by 11 species of wasps, 9 species of frugivorous/nectarivorous birds, and 2 species of phytophagus bats (Cox et al. 1992). Other animals on the islands include snakes, skinks, toads, and large land snails. Rats, pigs, cows, dogs, and cats have been introduced by humans. Feral pigs threaten native forest trees by destroying tree bark and disturbing seed banks of native species. Feral cats penetrate secondary and agroforest areas and prey on resident and migratory birds (S. Nelson, pers.obs.).

The Samoan islands have been inhabited by humans for approximately 3,000 years (Kirch and Hunt 1993, Petchey 2001). Today, the most serious environmental and social problem facing American Samoa is its rapid human population growth (Craig et al. 2000). The population estimate of 63,000 for the year 2000 is increasing at a rate of approximately 2.5% annually, which will result in a doubling time of only 28 years. A continued increase is expected given the high birth rate (4.5 children per female) and high proportion of pre-reproductives in the population; nearly 50% of the population is less than 20 years old (Craig et al. 2000). This exponential increase in the human population is coupled with a substantial degree of habitat loss due to the confines of a small oceanic island. Annually, 1-2% of rainforest is lost to agroforest in Samoa (Cole et al. 1988),

although current rates of forest loss may be higher. Land-use practices on Tutuila are largely influenced by the steep topography of the island. With 50% of the land area having a slope greater than 70%, there is relatively little land to use for agriculture and housing (Craig et al. 2000). As the human population expands, hillsides are being developed as are areas that were not formerly considered.

One area of refuge from development and human encroachment is the National Park of American Samoa. The National Park of American Samoa was officially established in September 1993 when a 50-year lease was signed between the National Park Service and the American Samoa Government representing the villages in the Park. The 9,000 acre park is spread out over three islands (Tutuila, Ta'u, Ofu), and includes 1000 acres that are underwater. The National Park of American Samoa contains many critical roosting sites for both species of fruit bats (Brooke 1998, Brooke 2001) and provides valuable native habitat for other species on the island (Freifeld 1999).

Pteropus tonganus

Three species of bats occur in the Samoan archipelago, *Pteropus samoensis*, *Pteropus tonganus*, and *Emballanura semicaudata* (Peale 1848, Andersen 1912). *E. semicaudata* is currently on the verge of local extinction in Samoa (Grant et al. 1994) or may already be extinct. The two pteropodid bat species found on the Samoan islands are the Samoan flying fox (*Pteropus samoensis* Peale), and the Tongan flying fox (*Pteropus tonganus* Quoy and Gaimard). The Samoan flying fox is solitary, diurnal, and prefers to forage on native forest fruits (Wilson and Engbring 1992, Thomson et al. 1998, Brooke et al. 2000, Brooke 2001). In contrast, the Tongan flying fox roosts in noisy colonies of thousands of bats, is primarily nocturnal, and forages both in native forest and agricultural areas (Quoy and Gaimard 1830, Banack 1996). Also called the white-

collared or insular flying fox, *P. tonganus* is a medium-sized fruit bat that weighs between ca. 300-600g, with a forearm length of 120-160 mm (Miller and Wilson 1997). Males are generally larger than females (Flannery 1995b). The fur is black or seal brown with a contrasting creamy yellow mantle (Miller and Wilson 1997).

The Tongan flying fox is common throughout the South Pacific and has the largest geographic range of any pteropodid species in Oceania. It is one of the most widely distributed of all *Pteropus* species (Koopman and Steadman 1995) and is found south of the equator from the Schouten Islands off NE New Guinea, south to New Caledonia, and east to the Cook Islands (Koopman 1993). The distribution of *P. tonganus* includes the easternmost limit for the Pteropodidae. The Tongan flying fox is described by Koopman (1979) as a "supertramp" species, referring to its absence from the largest and most species-rich islands and its prevalence on small, species-poor ones (Pierson and Rainey 1992, Mickleburgh et. al. 1992). There are few morphological differences among populations of *P. t. tonganus* separated by several hundred kilometers, although animals from Niue and the Cook Islands tend to be slightly smaller (Wodzicki and Felton 1980).

Tongan fruit bats roost colonially. Colonies can range in size from several individuals to several thousand. Roosts in coastal forest are in undisturbed areas on steep slopes immediately above the ocean or in upland forest (Brooke 1998). Within the roost, bats hang together in harem groups consisting of a single dominant adult male with two to sixteen adult females and their young, but group composition is highly labile (Banack 1996). Tongan fruit bat births have been observed year round in Samoa. The high number of pregnant and lactating females throughout the year suggests the ability of this species to rapidly increase its population size under optimal conditions (Banack 1996). The

mother carries her young until they are able to fly at 2-3 months of age (Brooke 1999). About 50% of copulations observed involved females that were nursing young, suggesting postpartum estrus in this species (Banack 1996).

Tongan fruit bats disperse from colonies to forage in native forests, agricultural areas, and residential areas (Banack1996). They are described as favoring agricultural areas, but the extent of use for each habitat type is currently unknown (Brooke 1998). They are phytophagus and visit numerous plants to consume fruit, nectar, pollen and leaves. A highly plastic forager, *Pteropus tonganus* uses 42 species of plants in American Samoa and has the ability to find food despite seasonal and distributional changes in food availability (Elmqvist et al. 1992, Banack 1998).

Mortality among Tongan flying foxes includes predation from raptors and snakes, disease epidemics, hurricanes, hunting by local people, and habitat loss (Brooke 1998). In many Pacific islands, fruit bats are considered a delicacy and consumed by local people, and were previously hunted to supply a luxury food trade (Craig et al. 1994a, Wiles et al. 1997). Archeological records indicate that *P. tonganus* has been hunted and eaten for at least the last 1,000 years (Steadman and Kirch 1990). In American Samoa, a ten year hunting ban was enacted to limit depredation (Craig and Syron 1992, Craig et al. 1994b, Brooke 2001). A current population estimate is 6300 or more Tongan fruit bats in American Samoa (A.P. Brooke, pers. comm.). Pierson et al. (1992) listed this species as priority grade II (not threatened) with priority status unknown.

Papua New Guinea and Kau Wildlife Area

Papua New Guinea comprises over 600 islands and includes the eastern half of the island of New Guinea, the two northernmost islands of the Solomon chain (Bougainville and Buka), and the Bismarck and Admiralty archipelagos. Papua New

Guinea lies between the Coral Sea and the South Pacific Ocean and is north of Australia. The country is a member of the British Commonwealth with Port Moresby as its capital. It is home to approximately five million people that speak over 800 languages.

Of the estimated 15,000 to 21,000 vascular plants found in Papua New Guinea, more than half are believed to be endemic (Mittermeier et al. 1997). Similarly, mammals are very diverse, with 242 species represented, 57 of which are endemic (Bonaccorso 1998). Bats, rodents and marsupials account for the bulk of the mammalian diversity, but bat species are the most numerous and highly diverse. Thirty-four species of Old World fruit bats are found in Papua New Guinea.

Fieldwork was done in the northern province of Madang in the Kau Wildlife Area. The forest at Kau Wildlife Area is an old and relatively undisturbed lowland rainforest tract of 300 ha that is an owned by the Dipida Clan. This area has been untouched by logging, shifting cultivation, burning, or hunting with firearms since 1963 when it was put aside by the clan for conservation. Laboratory work was conducted at the former Christensen Research Institute near Madang.

Blossom bats of Papua New Guinea

Both *Syconycteris australis* and *Macroglossus minimus* belong to the subfamily Pteropodinae (formerly Macroglossinae) (Kirsch et al. 1995) which reaches its maximum diversity in New Guinea (Flannery 1995a). Both *S. australis* and *M. minimus* have large geographic distributions and exhibit energetic plasticity. They are able to live in a variety of environments, including small islands, disturbed successional forests, lowlands, and montane rain forests (Bonaccorso and McNab 1997). *S. australis* is often described as a feeding and habitat generalist in Papua New Guinea, while *M. minimus* is a nectar specialist (Bonaccorso 1998) This is opposite of Australia, where *S. australis* is a nectar

specialist (Law 1992). Morphologically, the two species of bats are almost identical in linear size and body mass (approx. 15-20 g). They both have an elongated rostrum and a slender, protrusible tongue with brushlike projections to gather nectar and pollen. Their broad, short wings permit hovering and maneuverability (Gould 1978, Nowak 1999).

Syconycteris australis

The northern blossom bat, *S. australis*, resides in a variety of habitats, including lowland rain forest, dry sclerophyll woodland, montane/hill forest, and swamp forest (McKean 1972, Bonaccorso 1998). Its geographic distribution includes New Guinea and the east coast of Australia into New South Wales (Richards 1983, Bonaccorso and McNab 1997). This species weighs approximately 18 g and has an average forearm length of 39 mm (Law 1992, Bonaccoso 1998, Nowak 1999).

During the day, *S. australis* roosts singly in the foliage of trees and shows fidelity to day roost areas (Law 1996, Winkelmann et al. 2000). Northern blossom bats are excellent thermoregulators and are able to inhabit almost the entire range of elevations in New Guinea (McNab and Bonaccorso 1995). *S. australis* has a field metabolic rate that is double that predicted for an animal its size but a basal metabolic rate lower than predicted for its size (Geiser and Coburn 1999). An ability to undergo short periods of torpor may explain its extended distribution range and counteract unpredictable nectar availability and extended day roosting (Geiser et al. 1996, Coburn and Geiser 1998, McNab and Bonaccorso 2001).

S. australis is distinguished from *M. minimus* by a more robust dentition, which is better suited for feeding on fruits, but it also consumes nectar, pollen, and occasional insects (Bonaccorso 1998, Flannery 1995a). *S. australis* displays geographic variation in its diet within Australia. Law (1992, 1994) found *S. australis* to be a nectar specialist in

its range in southern Australia, but is a frugivore and partial folivore in the more northern part of its range. *S. australis* actively discriminated between sugar concentrations in concentration preference tests (Law 1993). Flower morphology may influence *S. australis* foraging, and result in resource partitioning within habitat areas (Nicolay and Dumont 2000). The northern blossom bat is abundant and ubiquitous in distribution throughout a wide range of habitat types and is listed as lower risk: least concern in the 1996 IUCN Red List of Threatened Animals (Baillie and Groombridge 1996).

Macroglossus minimus

Macroglossus minimus, also called the southern blossom bat, is the smallest of the blossom bats found in New Guinea. This bat weighs only 12-18g and has a forearm averaging 37.5 mm (Flannery 1995a, Nowak 1999). It is similar morphologically to *S. australis*, but is unique in its feeding habits and more limited in its distribution. *M. minimus* is a nectar specialist that occurs from sea level to 1000 m (Flannery 1995a, Bonaccorso 1998). Specimens from New Guinea lacked fruit in the stomach (McKean 1983), and captive individuals refused to eat fruit (Mickleburgh et al. 1992). Instead, *M. minimus* seems to prefer the nectar and pollen (Nowak 1999).

M. minimus is widespread in lowland New Guinea, where it is often found in secondary rain forest, hill forests, or coastal areas near mangroves (Mickleburgh et al. 1992, Bonaccorso 1998). It feeds in disturbed habitats and orchards due to its preference for domesticated bananas (Gould 1978, Bonaccorso 1998). This species roosts singly, in mother-infant pairs, or in small groups on the underside of large leaves, tree branches, or roofs of abandoned buildings (Bonaccorso 1998). Births can occur in any month, perhaps due to sperm storage (Hood and Smith 1989).

The southern blossom bat has a metabolic rate that is much lower than expected for a mammal of its body mass (Bonaccorso and McNab 1997). This species may be restricted to tropical areas because it can enter shallow daily torpor (Bartels et al. 1998). *M. minimus* is abundant and widespread throughout its range and is not currently threatened (Mickleburgh et al 1992); the small size and cryptic roosting may contribute to its listing as low risk: least concern in the 1996 IUCN Red List (Baillie and Groombridge 1996).

The Lubee Foundation, Inc.

The Lubee Foundation, Inc. was founded by the late Luis F. Bacardi in 1990 as a non-profit organization involved in the conservation of threatened and endangered species of Old World fruit bats. It maintains captive breeding populations and supports research of both captive and wild populations of bats at its facility near Gainesville, Florida. Bats are housed in outdoor circular flight exclosures that surround temperature-controlled roosting quarters. Eleven species of bats are currently housed at this facility, representing both fruit and nectar feeding bats. These species include *Cynopterus brachyotis, Eidolon helvum, Epomophorous wahlbergi, Glossophaga soricina, Pteropus giganteus, Pteropus hypomelanus, Pteropus poliocephalus, Pteropus pumilus, Pteropus rodricensis, Pteropus vampyrus,* and *Rousettus aegypticus*. In total there are over 600 fruit bats currently housed at the facility. My research at The Lubee Foundation, Inc. focused on *P. vampyrus*.

Pteropus vampyrus

P. vampyrus, also called the Malayan flying fox, is one of the world's largest flying foxes, and can attain a wingspan of 2 m. Members of this species weigh between 645-1092 g and have a forearm of 180-220 mm. *P. vampyrus* has a distinctively dog or

foxlike face, and pelage color ranges from mahogany or orange to black with a black, brown or grey/silver underbelly (Kunz and Jones 2000). The Malayan flying fox inhabits Burma, Thailand, the Phillipines, Sumatra, Java, Borneo, the Lesser Sundas, and adjacent islands (Andersen 1912, Medway 1969, Corbet and Hill 1992). There are currently seven recognized subspecies of *P. vampyrus* (Bastian et al. 2001).

The Malayan flying fox is found in a variety of habitats including primary forest, mangrove forests, mixed fruit orchards, and coconut groves (Medway 1969, Payne et al. 1985, Heideman and Heaney 1989). In Malaysia, *P.vampyrus* is most often found roosting in isolated and inaccessible areas such as mangrove forests and freshwater swamps (Payne et al.1985, Mohd-Azlan et al. 2001). Roosts are in the canopies of emergent trees and are often shared with *A. jubatus*. Mixed groups of these two species may range from 500 to 150,000 individuals (Mudar and Allen 1986, Heideman and Heaney 1989). Roosts abandonment is most often due to disturbance, habitat loss, or hunting (Mohd-Azlan et al. 2001).

Malayan flying foxes fly up to 50 km to reach their feeding grounds and shift feeding sites in response to changes in food availability (Medway 1969). They feed on flowers, nectar, and fruit, but most often on flowers and nectar (Gould 1977, Goodwin 1979, Payne et al. 1985). Pollen, nectar, and flowers of coconut and durian trees (*Durio zizebethinus*), fruits of rambutan (*Nephelium lappaceum*), figs (*Ficus* spp.) and langsat (*Lansium domesticum*) trees, in addition to fruits such as mangos and bananas are all preferred foods (Heideman and Heaney 1989, Kunz and Jones 2000) and are actively defended (Gould 1977). Figs are a dietary staple of *P. vampyrus,* while other foods are utilized on a more sequential basis throughout the year (Stier and Mildenstein 2001).

Female *P. vampyrus* give birth synchronously during a single annual peak, although the peak varies geographically and seasonally. Generally, females give birth between March and May to a single offspring, but twinning does occur (Medway 1969, Mickelburgh 1992). The gestation period is approximately 180 days (Kunz and Jones 2000). Young bats are carried by their mothers for the first few days, but later are left at the roosts while their mothers forage. Young suckle from their mothers for 2-3 months (Lekagul and McNeely 1977).

Camps of *P. vampyrus* in the Philippines once contained up to 100,000 bats (Mickleburgh et al. 1992, Nowak 1999). Unregulated hunting and habitat loss are the primary reasons for the decline in abundance for *P. vampyrus* (Mohd-Azlan et al. 2001). In many areas, this species is considered a nuisance because it feeds in fruit orchards (Medway 1969) and/or because of their noisy and conspicuous roosts (Kunz and Jones 2000). *P. vampyrus* is also hunted for local consumption and controls on hunting are considered unenforceable. As a result, their numbers have declined severely (Heideman and Heaney 1989). *P. vampyrus* is also threatened by the rapid loss and degradation of mangroves for coastal reclamation and aquaculture, and by commercial logging and land clearing for palm/rubber estates (Heideman and Heaney 1989). The Malayan flying fox is listed as a species that that may become threatened with extinction if trade is not regulated (Brautigam 1992)

CHAPTER 3
FRUIT CHOICE AND CALCIUM BLOCK USE BY TONGAN FRUIT BATS: DO FRUIT BATS SEEK OUT CALCIUM IN THEIR DIET?

Introduction

Diets of wild animals can be low in essential nutrients. When minerals are deficient in the diet, animals often seek out concentrated sources of these nutrients. These sources may include natural mineral licks and foods that are rich in the deficient mineral (Klaus et al. 1998). High sodium concentrations often attract wild animals to mineral licks (Belovsky and Jordan 1981, Moe 1993, Tracy and McNaughton 1995), although minerals such as calcium and magnesium may be equally important (Jones and Hanson 1985, Holl and Bleich 1987). When consumed by animals, these concentrated sources of minerals may help compensate for mineral deficiencies in the diet (Klaus et al. 1998).

The idea that animals preferentially select nutrient-rich foods or a nutritionally balanced diet from among a broad array or foods is coined "nutritional wisdom" and is highly controversial. When given a choice of diets, animals often chose foods with the highest nutrient content or minerals in which they were deficient (Ozanne and Howes 1971, Batzli and Pitelka 1983, Bromage and DeLuca 1984, Barclay 2002). However, other studies have found that animals failed to select foods that either met their dietary requirements or corrected for their nutritional deficiencies, but instead preferred palatable but nutritionally poor diets (Arnold 1964, Coppock et al. 1972, Muller et al. 1977, Oftedal and Allen 1996, Dierenfeld and McCann 1999, Zervas et al. 2001).

Selection for concentrated sources of minerals may be associated with the sex of the consuming animal. For example, adult females of many species were more likely to use mineral licks, especially during pregnancy and lactation (Faber et al. 1993, Montenegro 1998). It has been postulated that reproduction in bats may be limited by calcium deficiency (Barclay 1994,1995). To provide supplemental calcium to their diet, fruit bats on occasion seek out calcium-rich fruits and leaves (Barclay 2002).

This study was designed to explore whether wild Tongan fruit bats (*Pteropus tonganus*) sought out and preferentially consumed calcium when it was made available. Captive bats were tested as follows: (1) by documenting preference or avoidance of calcium-rich fruits and (2) by documenting use of commercial calcium blocks. It is hypothesized that bats would seek out calcium in their diet by consuming calcium-rich fruits and using the calcium blocks.

Methods

This study was conducted from December 2000 to August 2001 on the island of Tutuila, American Samoa (14° S, 170° W) in the South Pacific Ocean. The Tongan flying fox, *P. tonganus*, a medium-sized fruit bat (300-600 g), was used for this study. *P. tonganus* is common throughout the South Pacific (Miller and Wilson 1997) and is a feeding generalist that forages in both native forest and agricultural areas (Banack 1996).

Tongan fruit bats (13 males, 8 non-reproductive females, 2 (lactating) reproductive females) were captured in mist nets and transported to a 4 x 3 meter screened outdoor structure (the "bat house") to allow for movement and limited flight by the captive bats. Following a two-day acclimation period, bats were individually tested for five days, for a total of seven days in captivity. Bats were given twice their body mass in food nightly (wet weight) of a high and low calcium fruit suspended from plastic cable

ties on a large wooden dowel rod (the food bar). The types of fruit given to the bats varied every day depending on fruit availability on the island. The high-calcium fruits used in the experiments were 2-3 times higher in calcium than the low-calcium fruits (1.06-12.28 mg/g, 5.75 $\pm$ 1.39 mg/g for high-calcium fruits, 0.55-2.46 mg/g, 1.30 $\pm$ 0.59 mg/g for low-calcium fruits). Mineral concentrations for all native and agricultural fruits used in this study were based on previous mineral analyses (Nelson et al. 2000a). All fruit given to the bats were preferred foods of *P. tonganus* in American Samoa (Banack 1996). The most commonly used combination of fruits was local bananas and papaya, because they were readily available at local markets when native fruit species were unavailable. In addition to the high-calcium and low-calcium food types, a calcium block was suspended nightly from the food bar. The position of the calcium block was randomly assigned each night. The calcium block consisted of calcium sulfate and ground limestone, and contained 21-26% calcium (8 in1 Pet Products, Inc., Fairport Harbor, OH). Water and a salt lick were available *ad libitum.*

An infrared video camera (Sony Digital Handycam DCR-TRV 120) was placed in the bat house in front of the food bar each night to record fruit choices by the bat. The videotape recorded the first 1.5 hours of each nightly feeding session. Use of the calcium block was documented for each bat, as was the sequence of fruit choices. The first five fruit choices made by the bat were considered an indicator of fruit preference. The number of high-calcium fruits and low-calcium fruits chosen from the first five choices were analyzed statistically to document if bats sought out calcium in their diet. A binomial test was used to analyze preference for high or low calcium fruits (Hollander and Wolfe 1999).

To better understand preferences for fruit choice, I incorporated the effect of sugar and analyzed the relationship between calcium and sugar content using the multivariate technique Conjoint Analysis (Hair et al.1998). Conjoint analysis predicted bat choice for fruits when bats were given a subset of all fruits found on the island (see Green and Srinivasan 1978). Differences in sugar concentrations between samples of native and agricultural fruit were tested using a Mann-Whitney rank sum test (Sokal and Rohlf 1995). Sugar concentration of fruits was considered high if it was over 10%, intermediate if it was between 5-10% and low if it was less than 5% sugar. A sugar refractometer (Model # 300010, Sper Scientific, Scottsdale, AZ) was used to determine sugar values of different fruit types. High, medium and low calcium values were determined from previous mineral analysis of fruits (Nelson et al. 2000a). Both the sugar concentration and the calcium content of fruits were evaluated in Conjoint analysis to form part-worth estimates that were summed to totals. The largest part-worth totals resulted in high rankings for fruit preference.

Results

Choice of High-Calcium or Low-Calcium Fruits

Results of 63 trials and 146 h of video were analyzed to test whether bats preferred high or low calcium fruits when given a choice among them. The first five choices made by each bat in a trial were documented, resulting in a total of 262 choices. Low calcium fruits were chosen 200/262 times, and preferred 76% of the time. Low-calcium fruits were highly preferred over high-calcium fruits ($p < 0.001$). Fifty-five of the 62 times that high-calcium fruits were chosen, bats chose papaya. When papaya was removed from the high calcium fruit choice set, then low-calcium fruits were preferred

97% of the time ($p < 0.001$). Native fruit was chosen in the first five choices only once in 262 trials, by a bat that took a single small bite and then did not choose it again.

A native fruit (a fig, *Ficus tinctoria*) and an agricultural fruit (papaya, *Carica papaya*) were compared for sugar concentration using a Brix sugar refractometer. Papaya samples (n=12) averaged 12.6% sugar and were significantly higher in sugar ($p = 0.001$) than fig samples (n=15), which averaged 2.4% sugar. Although they provide an excellent source of calcium (O'Brien et al. 1998), figs were never consumed by bats and were highly avoided in this study. Despite using fresh, frozen, and three different species of figs (*Ficus tinctoria, F. unirauniculata, F. scabra*), figs were never eaten by the bats.

Results of a Conjoint analysis indicated the sugar content of fruit was the basis for fruit preference and selection by all of the bats that were tested (Table 3-1). Fruits that were the most preferred were high in sugar and low in calcium, and the least preferred fruits were low in sugar and high in calcium. Even if the sugar was high, high-calcium fruits were still avoided by bats. I then reanalyzed the data to evaluate female choice among fruits (Table 3-2). The results were very similar to those for the entire data set, indicating that individual females did not forage differently from the group, they avoided high-calcium fruits and preferred high sugar fruit. Sample sizes were too small to perform a Conjoint analysis for reproductive females.

Use of the Calcium Blocks

Twice as many females used the calcium blocks as did males (4 females, 2 males), and almost half (40%) of the females used the calcium blocks, including both reproductive females (Figure 3-1). Use of the calcium blocks by males was limited; only 15% of the males (2 subadults) used the blocks.

Table 3-1. Conjoint analysis results for all bats showing part-worth estimate totals and the resultant rankings of fruit characteristics to determine fruit preference by *P. tonganus*.

Sugar		Calcium			
Level	Part worth estimate	Level	Part worth estimate	Total	Ranking
High	0.27	Low	0.38	0.65	1
Medium	-0.1	Low	0.38	0.28	2
High	0.27	Medium	-0.05	0.22	3
Medium	-0.1	Medium	-0.05	-0.15	4
High	0.27	High	-1.57	-1.30	5
Low	-1.87	Low	0.38	-1.49	6
Medium	-0.1	High	-1.57	-1.67	7
Low	-1.87	Medium	-0.05	-1.92	8
Low	-1.87	High	-1.57	-3.44	9

Table 3-2. Conjoint analysis results for all females showing part-worth estimate totals and the resultant rankings of fruit characteristics to determine fruit preference by *P. tonganus*..

Sugar		Calcium			
Level	Part worth estimate	Level	Part worth estimate	Total	Ranking
High	0.27	Low	0.56	0.83	1
Medium	-0.09	Low	0.56	0.47	2
High	0.27	Medium	-0.27	0.00	3
Medium	-0.09	Medium	-0.27	-0.36	4
High	0.27	High	-1.41	-1.14	5
Low	-1.88	Low	0.56	-1.32	6
Medium	-0.09	High	-1.41	-1.50	7
Low	-1.88	Medium	-0.27	-2.15	8
Low	-1.88	High	-1.41	-3.29	9

Frequency of use was calculated as the number of times bats used the calcium lick divided by how many nights it was available to them. Frequency of use by males and females for the calcium blocks was very similar and did not exceed 10% (Table 3-3). Reproductive females used the calcium blocks with approximately four times the frequency (25%) of males or non-reproductive females. Most of the bats that used the calcium block were either reproductive females or subadults. In some cases, they used the calcium block before they ever chose fruit, and returned to use the calcium block intermittently while consuming fruit.

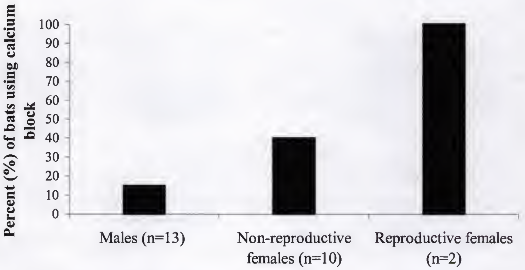

Figure 3-1. Calcium block use by male, female and reproductive female Tongan fruit bats. Thirteen males, ten females and two reproductive females were tested.

Table 3-3. Frequency of calcium block use by Tongan fruit bats.

Group	Number of bats in group	Total number of trials mineral used	Total days of trial	Frequency of use in the trials
Males	13	3	47	6%
Non-reproductive females	8	3	42	7%
Reproductive females	2	2	8	25%

Discussion

Tongan fruit bats did not consistently seek out concentrated sources of calcium by preferring calcium-rich fruits or by using commercial calcium blocks with high frequency. Instead, the bats in this study preferred fruits that were high in sugar and low in calcium. If high-calcium fruits were chosen, the fruit was usually papaya, a preferred fruit that is high in sugar. Females used the calcium blocks more often than males. Reproductive females used the calcium blocks at more than four times the frequency of non-reproductive females and males. Subadult males were the only males to use the calcium blocks.

Mammals often select foods to maximize their intake of carbohydrates like sugar (Provenza et al. 1996, Kimball et al. 1998), and fruit bats are no exception. Fruits high in sugars are highly preferred because they represent an important energy source for frugivorous bats (Dierenfeld and Seyjagat 2000b). Fruit bats prefer fruits such as papaya that are soft, succulent, and high in sugar, and will choose them over fruits low in sugar (Courts and Feistner 2000). Sugar, rather than calcium, appears to motivate dietary selection for fruits despite the importance of minerals to bat reproduction (Barclay 1995).

There may be a temporal component to resource use by bats. In other studies, high-energy fruits were consumed first by hungry and dehydrated bats emerging from the day roost, followed by consumption of mineral-rich leaves later in the night (Kurta et al. 1989, Elangovan et al. 2001). Tongan bats in this study had food available to them for ten hours a night, but only the first 1.5 h of feeding were recorded due to limited battery power and a lack of electricity in the bat house. This may have biased data collection toward documenting foraging for high-energy foods rather than calcium-rich foods. Thus, these results may not reflect feeding to consume deficient minerals that occurred later in

the night. Subsequent studies should record bat feeding at all times of the night to see if there is a temporal component to resource selection.

It was hypothesized that bats would use concentrated calcium sources such as calcium blocks to relieve mineral deficiencies. Overall, frequency of use was low for the calcium blocks, but noteworthy gender differences emerged. The only male bats to use the calcium blocks were two subadults. They licked the calcium blocks in almost half of their trials, and sampled high-calcium native fruits that were generally ignored by other bats. Subadult males may experience calcium deficiency due to rapid growth, and may ingest supplemental calcium to relieve temporary deficiencies.

Females, particularly reproductive females, used the calcium blocks in greater numbers and with greater frequency than did males. Twice as many females as males used the blocks, including use among all reproductive females tested. The two reproductive females used the calcium blocks at four times the frequency of either the males or non-reproductive females, and often used the calcium blocks before consuming any fruit. One of the lactating females removed high-calcium leaves from the *Callophyllum neo-ebuticum* (Clusaceae) tree in the cage and was seen consuming them at the food bar within 20 minutes of receiving sugar-rich agricultural fruits. Leaf consumption was also very high among reproductive females, resulting in supplemental calcium ingestion (Chapter 4). These results seem to indicate that in some cases, reproductive females may prioritize calcium ingestion over the ingestion of high-energy foods. This may indicate calcium deficiency among reproductive females. Unfortunately, very few reproductive females were caught, and their small sample size limits potential

interpretation of these data. Future work should examine food choice using larger numbers of reproductive females.

It is difficult to assess if animals in this study foraged with "nutritional wisdom." The assumption of this work was that calcium was the most deficient component of the diet, and would be pursued first by bats when foraging. Instead, the majority of *P. tonganus* fed on high-sugar, high-energy, agricultural fruits soon after their presentation, potentially to maximize energy consumption. Although female Tongan bats were potentially deficient in calcium (Barclay 1995), they did not choose high-calcium fruits from among those offered. A limited number of bats did seek out minerals by using the calcium blocks. Pregnant females and rapidly-growing subadult bats used the calcium blocks the most, and were the most likely candidates for calcium deficiency.

Factors that motivate fruit selection among Tongan fruit bats warrant further research, with experiments that include a larger number of reproductive females, and observations of feeding done at different times throughout the night. This may create a more complete picture of nutritional priorities and how they affect temporal patterns of resource use.

CHAPTER 4
FOLIVORY IN FRUIT BATS: ARE LEAVES A NATURAL CALCIUM SUPPLEMENT?

Introduction

Folivory, or leaf-eating by bats, is a well documented phenomenon (Marshall 1985, Lowry 1989, Funakoshi et al. 1993, Kunz and Ingalls 1994, Kunz and Diaz 1995, Banack 1996, Tan et al. 1998, Ruby et al. 2000). Leaves are an important dietary source of minerals, carbohydrates, and protein, and are especially rich in calcium (Tan et al 1998, Nelson et al. 2000b, Ruby et al. 2000,). Leaves are a consistent food source for bats; they are available year-round and are predictable in time and space (Kunz and Ingalls 1994, Rajan et al. 1999). Thus, leaves may provide a greater net return per foraging bout than ingestion of large amounts of low-protein fruit or the active pursuit of insects (Thomas 1984, Kunz and Ingalls 1994, Tan et al. 1998). In addition, steroid hormones found in leaves may influence bat reproductive activity (Wickler and Seibt 1964, Kunz and Diaz 1995).

Bats consume leaves by leaf-fractionation. This process includes masticating the leaves into a bolus, swallowing the liquid portion, and ejecting the flattened fibrous pellet (Lowry 1989, Funakoshi et al. 1993, Kunz and Ingalls 1994, Kunz and Diaz 1995). By rejecting the fibrous portion, bats are able to consume leaf nutrients without altering their digestive tract or increasing wing loading (Kunz and Ingalls 1994). Frugivorous bats appear to be pre-adapted for folivory by leaf fractionation; their dentition and gut morphology are specialized for extracting and digesting a largely liquid diet (Tedman and

Hall 1985, Kunz and Ingalls 1994). To shift their diet alternately between one of fruits to leaves would involve little, if any, change in form of function of the gut or dentition (Kunz and Diaz 1994).

Folivory was once thought to be rare among fruit bats, with leaves taken only when other food sources were scarce (Marshall 1985, Funakoshi et al. 1993, Pierson et al. 1996). However, recent studies have shown that leaf-eating is both common and widespread among Old World flying foxes (Banack 1996, Tan et al. 1998, Ruby et al. 2000). Folivory has been reported for at least 17 species of Old World Megachiroptera, and leaves eaten by bats include 44 species of plants represented by 23 families (Kunz and Diaz 1995). Bats locally consume a large variety of leaves. For example, *Cynopterus brachyotis* fed regularly on the leaves of 14 plant species in southern India (Tan et al. 1998), and *Pteropus dasymallus* on nine species in Taiwan (Funakoshi et al 1993). The incidence of leaf pellets under feeding roosts in Taiwan was 37-50%, and occurred almost throughout the year (Funakoshi et al 1993). However, this may be an underestimate. Leaf pellets are often not noticed because they are inconspicuous among other plant material on the forest floor (Kunz and Ingalls 1994).

Calcium is of particular interest in bat biology (Barclay 1994,1995, Kunz et al. 1995, Bernard and Davison 1996). It has been proposed that females may be stressed for calcium due to the mineral demands of both pregnancy and lactation (Barclay 1994,1995). To compensate for the large size of their offspring, bats donate their own skeletal calcium to build the bones of their young (Barclay 1995). Leaves represent a rich and consistent source of calcium to bats that are mineral stressed. Calcium concentrations are often much higher in leaves than in fruit (Nelson et al. 2000b, Ruby et al. 2000).

While some fruits may be high in calcium, it is not readily available if the Ca:P ratio is less than the optimum of 2 to1 (McDowell 1992, Robbins 1993). The Ca:P ratio is three times higher in leaves than in fruits, which further suggests that leaves may be valuable for their high calcium content (Kunz and Diaz 1995, Ruby et al. 2000).

This study examined if folivory among a sample of captive, wild-caught Tongan fruit bats (*Pteropus tonganus*). This study is the first to examine the amount of leaves that are consumed by individual fruit bats in a single night, and to calculate how much calcium folivory contributes to total daily calcium intake. I also describe gender and age differences in leaf consumption and explain an observed pattern of leaf eating.

Methods

Research was conducted from December 2000 to August 2001 on the island of Tutuila, American Samoa (14° S, 170° W) in the South Pacific Ocean. All 23 (13 male, 10 female) Tongan fruit bats (*Pteropus tonganus*) were caught using large mist nets and transported to the "bat house." The bats consisted of four adult male, nine juvenile male, four adult female, and six juvenile female bats. Two of the adult females were lactating. The bat house was a 4 x 3 m outdoor wooden structure with an adjoining 4 x 3 m screened outdoor pen specifically built to house bats for these experiments. The outdoor pen contained a single *Callophyllum neo-ebudicum* (Clusiaceae) tree for roosting and leaf consumption (Trail 1994, Whistler 1994). This tree was the only leaf source for the bats in the present study. The bats could fly and move easily within the outdoor enclosure. Each night, bats were offered twice their body mass in confirmed bat foods from the island (Banack 1996). The fruit type varied each day depending on fruit availability on the island. See Chapter 3 for further details. Salt rings comprised of salt and mineral oil and contained 96-99 % salt (Pet Products, Inc., Hauppauge, N.Y.) as well as collected

rainwater were available to the bats *ad libitum*. Feeding trials were conducted on individual bats and lasted three to five days following a two day acclimation period, for a total of seven days in captivity. Only one bat was present and tested at a time in the outdoor enclosure. Fruit traps, or raised screen platforms, covered the ground of the enclosure to catch food and leaves dropped by the bats while they were feeding.

The number of leaves and percentage of leaf eaten were recorded for each bat daily. Limited samples of representative leaves were collected and dried at 105°C for 24 h. Leaf samples included both whole leaves and leaves partially eaten by bats. Samples were analyzed at the University of Florida in Gainesville, Florida USA. Dried samples were prepared and digested according to Miles et al. (2000). Calcium concentrations (ppm) were assessed by atomic absorption spectrophotometry (Perkin-Elmer AAS 5000 Norwalk, CT.). All values were calculated on a dry matter basis.

Males and females, and juveniles and adults, were compared to identify differences in leaf-eating behavior. The bats seemed to fall into three general categories from visual observation of Table 4-1. Bats that habitually consumed leaves more than 50% of the days they were in the pen were classified as habitual leaf-eaters. Some of the bats consumed leaves occasionally, less than half of the days that they were in the experiments, and were classified at occasional leaf-eaters. Some bats never consumed leaves and were therefore classified as non-leaf eaters. These three groups were later compared for total calcium consumption.

Supplementary calcium values for each group were calculated as the total amount of leaf matter eaten by a bat multiplied by the calcium (Ca) concentration of the leaves (8861.47 mg/g Ca). To determine how much calcium folivory contributes to total daily

Table 4-1. Age and gender of bats used in the experiment, and number of leaves, amount of total leaf matter eaten (g), calculated calcium supplement (mg) gained by leaf-eating.

Gender	Age	Leaf eater?	Days leaves eaten (%)	Total leaves eaten	Total leaf matter eaten (g)	Calcium supplement (mg)
Male	Adult	yes	50	5	1.45	12.85
Female	Juvenile	yes	20	0.75	0.3	2.66
Female	Adult	yes	100	4	1.07	9.47
Female	Adult	yes	100	7	1.82	16.13
Male	Juvenile	yes	100	26	6.76	59.91
Male	Juvenile	yes	100	12	3.12	27.65
Male	Juvenile	yes	33	1	0.26	2.31
Female	Juvenile	yes	25	2	0.52	4.61
Female	Juvenile	yes	40	3	0.81	7.18
Male	Juvenile	yes	100	8	2.08	18.43
Male	Juvenile	yes	60	12	3.12	27.65
Male	Juvenile	yes	60	10	2.6	23.04
Female	Adult	yes	67	2	0.55	4.87
Male	Adult	yes	100	14	3.64	32.26
Female	Adult	yes	67	4	1.04	9.22
Male	Juvenile	yes	80	11	2.86	25.34
Male	Adult	yes	50	2	0.52	4.61
Male	Juvenile	yes	50	4	1.07	9.48
Female	Juvenile	yes	25	1	0.26	2.31
Male	Adult	no	0	0	0	0
Female	Juvenile	no	0	0	0	0
Male	Juvenile	no	0	0	0	0
Female	Juvenile	no	0	0	0	0

calcium intake, I calculated the average amount of supplemental calcium consumed for each of the three groups, and compared that to the average total calcium ingested by that group. Differences in consumption of leaves between gender and age were compared using two-tailed *t* tests. The Kolmogorov-Smirnov test was used to evaluate assumptions of normality for each variable, and Levene's test was used to evaluate the assumption of

normality between groups (Sokal and Rohlf 1995). Total calcium consumption for the three leaf-consumption groups were compared using a one way ANOVA.

Results

Ninety-four feeding trials were performed on 23 Tongan fruit bats. Leaves were consumed by 82.7% of the bats in this study. More males (92%) consumed leaves than females (70%). The total leaf mass eaten (g) differed ($p = 0.02$) between the sexes; males consumed an average of 9.55 $\pm$ 6.99 SD leaves, and females consumed an average of 2.97 $\pm$ 2.04 leaves over the period of the feeding trial. This resulted in 22.14 $\pm$ 16.00 SD (g) of additional calcium for males and 7.06 $\pm$ 4.56 SD (g) for females. The amount of additional ingested calcium was different ($p = 0.02$) between males and females. Juvenile male and juvenile female bats differed ($p = 0.04$) in their consumption of leaves (24.22 $\pm$ 17.03 and 4.19 $\pm$ 2.24, respectively); but male and female adult bats did not ($p = 0.40$). Twice as many juvenile males ate leaves than juvenile females (8 males: 4 females).The maximum number of leaves eaten in a single night (26) was by a young male.

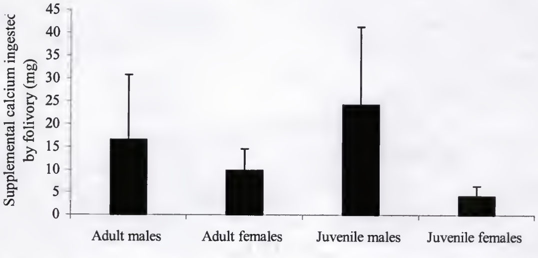

Figure 4-1. Supplemental calcium ingested (mg/g) by folivory for adult and juvenile males and females.

The number of leaves eaten by individuals over a five-day period ranged from 0.75 to 26 leaves. Overall, folivory provided 2.3 to 32.26 mg of additional calcium to the diet of fruit bats in the present study. There were similar numbers of habitual and occasional leaf eaters (11 and 8, respectively); however, habitual leaf eaters consumed significantly more calcium through leaf-eating than occasional leaf eaters ($p = 0.04$). Habitual leaf-eaters consumed an average of 10 $\pm$ 6.56 SD leaves, which contributed an additional 23.89 $\pm$ 15.05 SD mg/g of dietary calcium (Figure 4-2). This represented an average dietary increase in calcium of 11-46% when compared to the daily calcium consumption for each bat in that group. Occasional leaf-eaters consumed an average of 2.34 $\pm$ 1.54 SD leaves, which added an additional 5.75 $\pm$ 3.82 g of calcium to their diet. Occasional leaf-eating represented an average dietary increase in calcium of 3-22% when compared to the daily calcium consumption for each bat. Non-leaf-eaters ($n = 4$) added no additional calcium to their diet.

When eating leaves, *P. tonganus* often avoided the fibrous midrib, and instead ate around it (Figure 4-3). I chemically analyzed portions of a leaf cut along the midrib that did not contain the midrib to imitate leaf consumption by bats. These samples were compared to whole leaves that contained the fibrous midrib. The portions with the midrib contained 15.08 mg/g of calcium, and leaves without the veins contained 8.86 mg/g of calcium. Unfortunately, the work was largely exploratory and several leaves were used to produce only one analyzed sample for both the leaves containing the midrib and those that did not contain the midrib. Thus, a statistical analysis of this sample was not possible.

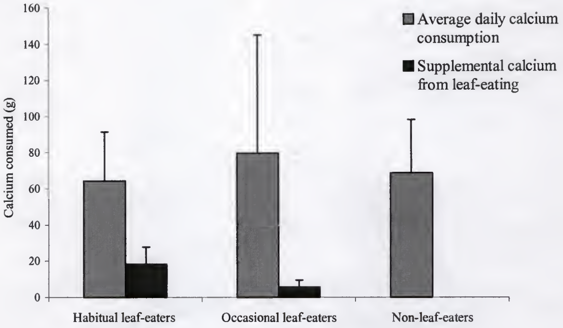

Figure 4-2. A comparison of total calcium ingested among habitual, occasional, and non-leaf-eaters. Total calcium is average daily calcium and supplemental calcium from leaf-eating combined. Total average daily calcium values for each group are calculated from values found in Chapter 5.

Figure 4-3. Typical pattern of leaf consumption by *Pteropus tonganus* in American Samoa.

Discussion

Previous studies of folivory (Marshall 1985, Lowry 1989, Funakoshi et al. 1993, Kunz and Ingalls 1994, Kunz and Diaz 1995, Banack 1996, Tan et al. 1998, Ruby et al.

2000) were based on indirect means of quantifying leaf-eating by bats and thus were unable to describe either the amount of leaves eaten per bat, or the sex and age of the leaf consumer. This study quantified both the amount of leaves consumed by individual bats and how much folivory contributed to total dietary calcium intake. The majority (83%) of wild-caught bats engaged in leaf-eating while in captivity. Both sexes consumed leaves, but male bats consumed more leaves than females, and juvenile males consumed both the greatest number and volume of leaves of all groups. Habitual leaf-eating bats could potentially increase their dietary calcium consumption by 46%. Clearly, folivory is both widely and frequently practiced by *P. tonganus*, and it has the potential to contribute significantly to the total amount of ingested calcium.

The assumption in conducting this study was that the brevity of the time that bats spent in captivity would neither alter nor adversely affect the current mineral status of the bats used in the experiments. That is, the deficiencies or excesses of their native diet would influence their consumption patterns while in captivity. If this is true, then both male and female bats consume leaves as a regular part of their diet in the wild, but consumption patterns and volume of leaves eaten differs among sexes and groups.

Some bats consistently consumed leaves each day of the experiment, while others only sampled the leaves intermittently. An interesting outcome of this analysis is that although the average daily (dietary) calcium ingestion was lower for habitual leaf-eaters than for occasional leaf-eaters, both groups were appeared similar in the total amount of calcium ingested. Habitual leaf-eaters exhibited the greatest variation in daily dietary calcium ingestion. Non-leaf eaters ingested the least calcium, but calcium ingestion by

non-leaf-eaters was very similar to the average daily calcium ingested by the habitual leaf-eaters.

Kunz and Diaz (1995) observed only mature males carrying leaves, and hypothesized that folivory may be limited to adult male bats. Overall, adult males consumed significantly more leaves and ingested more leaf matter than adult females in this study. Interestingly, leaf-eating was practiced most often by juvenile males. Other work has suggested that compounds extracted by leaf fractionation could be possible regulators of reproductive activity for bats (Kunz and Ingalls 1994, Kunz and Diaz 1995). *Erythrina* leaves may contain one or more metabolites (alkaloids) important for reproduction, and they are consumed by *P. tonganus* on the island of Tonga (Harris and Baker 1959). Perhaps, in addition to being a rich calcium source that supports rapid growth, leaves may also influence reproductive activity in young male bats.

Female bats consume leaves to access nutrients and minerals not available in fruits (Kunz and Diaz 1995). Leaves analyzed from American Samoa were very rich sources of calcium and other macrominerals. Leaves contained concentrations of many nutrients comparable or higher than those of ripe fruit preferred by fruit bats in Samoa (Nelson et al. 2000b). Leaves tended to have higher levels of calcium, sodium, manganese, and magnesium than ripe native or agricultural fruits (Nelson et al. 2000b). Also, leaves are widely available in both the wet and dry season (Whistler 1994). Thus, leaves may represent a rich, year-round, readily accessible source of concentrated minerals for female bats.

P. tonganus females in Samoa may also consume leaves for their high calcium content. Banack (1996) found that female Tongan fruit bats in American Samoa gave birth year-round, and young were seen on mothers all months of the year. She also observed copulations with pregnant females, suggesting that the female was nursing while allocating her own calcium for the skeletal formation of a new offspring (Barclay 1995, Banack 1996). Both gestation and lactation are nutritionally demanding, and their combined effect may promote leaf-eating in females. In addition, the population of *P. tonganus* in American Samoa has increased 3-fold over the last decade, following a series of three destructive hurricanes that decimated the bat population (Craig et al. 1994b, Elmqvist et al. 1994, Brooke 1998). This population's rapid expansion may have resulted in additional calcium stress to reproductive females. However, despite this potential calcium stress, *P. tonganus* in American Samoa consistently chose low-calcium, high-sugar agricultural foods that resulted in inadequate calcium consumption, and high levels of retention that suggest calcium stress (Chapter 5). All together, the cumulative demands of gestation and lactation, overlapping generations, a rapid population increase, and a diet low in calcium, may promote in leaf-eating by female bats.

Recent evidence indicates that leaves are consumed by bats throughout the year. There are reports of year-around leaf consumption based on either analysis of fecal remains, leaf parts discarded beneath roosts (Lowry 1989, Parry-Jones and Augee 1991a, Bhat 1994), or direct observation (Zortea and Mendes 1993). Bhat (1994) noted that leaf-eating was common in each month of the year in *C. sphinx.* Banack (1996) described year-round leaf use by both *Pteropus samoensis* and *P. tonganus* in American Samoa. This study is consistent with her findings; leaves were consumed in each of the eight

months of this study, with no apparent differences between months or seasons. However, the manner in which Tongan fruit bats consumed leaves suggests that they are actively avoiding the fibrous midrib, despite it being a rich source of calcium. Bats may avoid the midrib because it has high levels of tannins or secondary compounds (Dasilva 1994).

This study has shown that males consumed significantly more leaves than females, and that folivory can contribute significantly to the total dietary calcium of leaf-eating bats. However, the motivation for folivory still cannot be ascribed to a single factor; both the high calcium content and presence of hormonal compounds in leaves may play integral roles. The propensity of males to consume large amounts of leaves suggests a hormonal motivation, but the calcium contribution to the diet due to folivory is significant and noteworthy. Future research should test whether hormonal compounds are present in the *Callophyllum neo-ebudicum* leaves and if these compounds influence reproductive cycles in this bat. Meanwhile, leaf-eating is a common practice among Tongan fruit bats, and the leaves provide a rich and concentrated calcium supplement to the often calcium-poor diet of fruit bats.

CHAPTER 5
BIOAVAILABILITY AND APPARENT ABSORPTION OF MINERALS CONSUMED BY WILD TONGAN FLYING FOXES IN AMERICAN SAMOA

Introduction

Previous research on nutrition in bats has concluded that energy and protein are the most important dietary nutrients (Thomas 1984, Herbst 1986, Fleming 1988). Although dietary mineral composition and concentration are not often measures of dietary quality (Cole and Batzli 1979, Batzli 1986), recent studies have illustrated the importance of minerals in bat nutrition (Uhland et al. 1992, Barclay 1994,1995,). Nutrients that are consumed at marginal or inadequate levels with respect to requirements may limit animal performance (Oftedal 1991). Mineral nutrition can affect fecundity, number of litters, and survival of offspring (Batzli 1986, Delgiudice 1990) and are critical to the basic physiological functions of animals (McDowell 1992). Despite their importance to survival and fecundity, mineral requirements remain largely unknown for most species.

Calcium is the most abundant mineral in the body, and one of interest in bat biology. For a pregnant or lactating female, inadequate calcium intake causes weakened bones and low milk production (McDowell 1992). For her dependent young, inadequate calcium from the mother results in inhibited growth, loss of body mass, and reduced mineralization of bone that can result in lameness and bone fractures to young bones (Radostits et al. 1994). Low dietary intakes also affect successive generations; offspring of rats fed a poor calcium diet survived but could not reproduce and had only 75-80% of

the normal skeletal calcium content (Brommage and DeLuca 1984). The excessive mineral demands of pregnancy and lactation result in a negative calcium balance as females donate their own skeletal calcium reserves to build the skeletons of their young (Radostits et al. 1994, Bernard and Davison 1996). Raising several young in sequential years may result in osteoporosis in females, resulting in bones that fracture and easily break (Keeler and Studier 1992, Studier et al. 1994a).

Requirements for calcium and all other minerals needed to maintain the health of bats are currently unknown. To examine mineral levels in bats, previous studies have used indirect methods to quantify minerals in the diet. Methods of calcium status evaluation have included analysis of blood plasma (Kunz and Stern 1995, Heard and Whittier 1997, Dierenfeld and Seyjagat 2000a, Kwiecinski et al. 2001), whole body mineral composition (Studier 1994, Studier and Kunz 1995), and fecal analysis without a knowledge of the types or quantities of foods consumed in the diet (Studier et al. 1991, Keeler and Studier 1992, Studier et al.1994b). However, none of these methods quantified the amount of minerals consumed, absorbed, or the absorption efficiency of minerals. Together, these factors can create a portrait of bat feeding and demonstrate the degree of mineral inadequacy in an individual.

The present study was designed to determine mineral absorption efficiencies of flying foxes using apparent absorption. Apparent absorption has been used in a limited number of nutritional studies (Belovsky and Jordan 1981, Dierenfeld and Seyjagat 2000b), but not on wild bats. This method measures both mineral intake and fecal excretion, and it can account for the unique manner of feeding by fruit bats. To feed on fruit and leaves, fruit bats chew the plant matter into a fibrous pellet, swallow the juice,

and expel the flattened pellet (Lowry 1989, Kunz and Ingalls 1994, Kunz and Diaz 1995). Previous studies were not able to account for minerals found in the expelled pellet.

Due to a lack of mineral requirement standards for fruit bats, target levels for nutrients are based on estimated requirements reported for other mammals (NRC 1995), or what is fed in captivity to maintain breeding populations (Courts 1998). Previous research has compared values either to those of domestic laboratory mammals or to a generalized mammalian standard (Oftedal and Allen 1996, Dierenfeld and Seyjagat 2000a). Mineral requirements established for rats and primates seem the most appropriate models to compare to fruit bats in the absence of true bat values. Rats are similar in size to bats and are monogastric, whereas primates are larger but share a monogastric gut, similar feeding habits, and perhaps an evolutionary past with bats (Pettigrew 1991).

No studies have confirmed that bats are significantly different than other mammals in their general nutrition needs, but differences in absorption of minerals can occur among similar species, and dietary diversity is high within the family Pteropodidae (Walinski and Guggenheim 1974, Marshall 1985, Courts and Feistner 2000). Nutrient requirements are affected by such factors as growth rate, reproductive output, and metabolic needs (Oftedal and Allen 1996). These factors can be markedly different for rats and primates when compared to bats. Moreover, published requirements for laboratory animals are often in excess of true requirements to allow for ingredient variation and other margins of safety (Oftedal and Allen 1996). Thus, mineral requirements of domestic animals may be an inappropriate standard for fruit bats.

The Tongan flying fox, *Pteropus tonganus* (Quoy and Gaimard, 1830) was used in this study. *P. tonganus* is one of the most widely distributed of all *Pteropus* species

(Koopman and Steadman 1995) and has adapted to many habitats and food types. It is a highly plastic forager and feeds on both native and agricultural fruits (Banack 1996, Pierson and Rainey 1992). Its wide geographic range and use of both native and agricultural fruits suggests a highly generalized digestive system. Additionally, the Tongan fruit bat is a mid-size fruit bat (300-600 g; Miller and Wilson 1997) so mineral absorption values are applicable to a wide range of body sizes. Overall, the Tongan fruit bat is an excellent study species because results should be widely applicable to many other flying fox species.

This study used apparent absorption to quantify mineral ingestion and absorption in *P. tonganus*. Mineral absorption results were compared to both mammalian standards and other fruit bat mineral retention values to quantify if Tongan fruit bats met recommended mineral standards or were mineral stressed as determined by elevated absorption levels. This was the first attempt to quantify ingestion, absorption, and retention for minerals in wild populations of flying foxes.

Methods

Netting and Housing of Bats

Research was conducted from September 2000 to August 2001 on the island of Tutuila, American Samoa (14° S, 170° W) in the South Pacific Ocean. Tongan fruit bats (13 males, 10 females) were captured in large mist nets (6-18 m, 4 inch mesh, Avinet, Inc.) attached to pulleys set high in coconut trees or on tall poles. Nets were raised at sunset and were checked every 30 minutes until midnight when they were taken down. Netting was conducted all over the island so that the bats used in the mineral retention trials would represent the bat population of the entire island.

Following capture, a single bat was transported to the "bat house," a 4 x 3 m wooden building with an adjoining 4 x 3 m screened outdoor structure. Both structures were built to temporarily house bats for these experiments. The outdoor structure was a wooden frame enclosed with rat wire and screen, and was lined with fishing nets to facilitate roosting and movement of bats. It contained a single *Callophyllum neo-ebudicum* (Clusiaceae) tree that could be used by bats for roosting and leaf consumption. The bats could fly and move easily within the outdoor structure. Water and salt licks were available to the bats *ad libitum* inside the outdoor structure at all times. Calcium blocks were occasionally available to bats as part of another experiment (Chapter 3). Salt licks consisted of salt and mineral oil, and calcium blocks consisted of calcium sulfate and ground limestone (8 in1 Pet Products, Inc., Fairport Harbor, OH).

Mineral Metabolism Experiments

Bats were sexed, weighed, measured, and examined for injuries upon capture. Following a two day acclimation period, the bats were tested for five days in the bat house. Each bat spent a total of seven days in captivity and only one bat was present and tested at a time in the outdoor structure. Bats were given twice their body mass in food nightly (wet weight) so that hunger would not result in atypical food choices. To test if Tongan fruit bats would choose high calcium fruits if they were available (Chapter 3), each bat was presented nightly with equal masses of one high-calcium fruit and one low-calcium fruit. The fruit type varied each day depending on fruit availability on the island. Native fruits were typically high in calcium and agricultural fruits were low in calcium (Nelson et al. 2000a). The high-calcium fruits used in the experiments were 2-3 times higher in calcium than the low-calcium fruits (1.06-12.28 mg/g, 5.75 ± 1.39 mg/g for high-calcium fruits, 0.55-2.46 mg/g, 1.30 ± 0.59 mg/g for low-calcium fruits). Mineral

concentrations for all native and agricultural fruits were based on previous work analyzing the mineral concentrations of bat fruit in Samoa (Nelson et al. 2000a, 2000b). According to Banack, all fruits given to the bats were preferred *P. tonganus* foods in American Samoa (Banack 1996, 1998).

During fruit preparation and handling, disposable plastic gloves were worn, and stainless steel or plastic utensils were utilized to avoid mineral contamination. Utensils were washed using 1:1 vinegar and distilled water solution. Fruits were cut into equal-sized cubes, weighed, and suspended on plastic cable ties from a large wooden dowel rod (the food bar). Desiccation factors were determined from sub-samples of both high and low-calcium fruit, handled and prepared in the exact same way as the fruits given to the bats, but placed in a separate small cage within the outdoor structure each night. The representative fruits were weighed the next morning to establish a desiccation factor for each fruit type that was later subtracted from the samples to yield an accurate estimated wet weight value. Fruit traps, or raised screen platforms, covered the ground of the outdoor structure to catch food and leaves dropped by the bats while they were feeding. The fruit traps were washed daily with collected rainwater.

All samples were collected the following morning. Food remains were separated into two categories: uneaten food still hanging from the food bar or dropped to the fruit traps below (hereafter called orts), and food that had been chewed on and sucked of all its juice and then spit out as small flattened disks (called ejecta). Fecal matter, representative samples for both fruit types, and partially eaten leaves were also collected, separated, and weighed. A wet mass was recorded for each type of sample (orts, ejecta, fecal, representative fruit, and leaves) for each day. Seeds were removed from all fruits before

weighing. Samples were placed in a drying oven for 24 hours at 105° C and dried to a constant mass and reweighed. Urine was not collected separately because it is not used for apparent absorption (McDowell 1992). All samples were stored in plastic bags in airtight plastic containers containing desiccant. The containers were stored in an air-conditioned laboratory to prevent mold growth until laboratory analyses were done.

Analysis of Samples

Samples were analyzed at the Animal Nutrition Laboratory at the University of Florida in Gainesville, Florida. Dried samples were weighed and dry ashed at 550°C for 12 hours. Samples were prepared and digested according to the procedures of Miles et al. (2001). Mineral concentrations of Ca, Cu, Fe, Mg, Fe, Mn and Zn were assessed using flame atomic absorption spectrophotometry using a Perkin-Elmer AAS 5000 (Perkin-Elmer 1980) after wet digestion in HCL and dilution in 1% lanthanum solution. Phosphorus was measured separately using a colorimetric assay (Harris and Popat 1954). Samples were analyzed in duplicate if sample size allowed. Standard reference material (citrus leaves 1572, National Institute of Standards and Technology, Gaithersburg, MD.) was run with each sample set.

Data analyses included calculating the total mineral consumption by each bat for each day. The mineral concentration numbers of fruit were multiplied by the amount of fruit that was consumed each day. This resulted in a value for total mineral intake.

Apparent absorption was calculated using the following equations. Mineral calculations were calculated on a dry matter basis. Each calculation was performed for each mineral, for each bat, for each day they were housed in the outdoor structure, resulting in 805 apparent absorption values. The average absorption value for each bat, for each mineral, for all days in captivity was averaged and compared.

Total mineral intake (g) =
(total amount of fruit offered (g)) – (total amount of orts (g)) – (total amount of ejecta (g))

Apparent mineral absorption (%) =

$$\frac{\text{(total mineral intake)} - \text{(fecal mineral)}}{\text{(total mineral intake)}} \times 100$$

Statistical Analysis

To statistically analyze the data set, mineral apparent absorption values among all bats were tested for normality using the univariate Shapiro-Wilkes test. The values were not normally distributed, so the data were rank-transformed (Sokal and Rohlf 1995). The transformed data were analyzed using Principal Components Analysis (PCA) to evaluate patterns of variation between different bat sexes, ages, and reproductive states of the bats. PCA assesses relationships of independent variables within a single data set and places factors that are ecologically similar in close proximity in ordination space (Garigal et al. 2000). Comparisons of apparent absorption values for each mineral between *P. tonganus* and the giant flying fox *Pteropus vampyrus* (Chapter 7) were analyzed using a two sample t-test assuming equal variance (Sokal and Rohlf 1995).

Results

Mineral Consumption

A total of 115 feeding trials were performed on 23 wild Tongan fruit bats. The bats consisted of four adult male, nine juvenile male, four adult female, and six juvenile female bats. Two of the adult females were lactating. When all values for average mineral absorption values were analyzed, PCA resulted in no clear patterns or trends for either the macrominerals or trace elements (Table 5-1). Subsequent t-tests failed to distinguish clear patterns among the data for different sexes, ages, and reproductive classes for all

minerals. Thus, data were grouped and evaluated as a single data set for all future analyses.

Table 5-1. Results of the principal components analysis comparing average mineral absorption values for all bats.

	Factor 1	Factor 2
Eigenvalue	3.33	1.26
Percent variation explained	47.50	17.98
Contributions of each individual variable		
Calcium	0.45	0.26
Phosphorus	0.32	0.52
Magnesium	0.34	0.14
Zinc	0.29	-0.69
Iron	0.28	0.24
Manganese	0.48	-0.12
Copper	0.42	-0.32

The results of the PCA for mineral components identified two factors with eigenvalues > 1 that together explained 65.48% of the variation. Factor 1 loaded positively for all minerals, but the factor scores were highest for calcium, manganese and copper. Factor 2 loaded positively for all minerals but zinc, manganese, and copper. Phosphorus loaded most heavily and positively of all the minerals in Factor 2 (0.52), and calcium was the second highest with the factor score 0.26.

Bats were offered an average of 566.7 $\pm$ 109.30 g (wet weight) of food per day of which an average of 332.2 $\pm$ 113.00 g was consumed. Tongan bats consumed 85% of their body mass daily on a wet matter basis and 17% on a dry matter basis. Despite the large amount of food rejected daily, both the amount of food offered and the amount consumed consistently failed to meet the required mineral standards established for laboratory animals (Table 5-2). Calcium (Ca), phosphorus (P), manganese (Mn), and copper (Cu) were below the required levels for both the diet offered and consumed. Zinc

(Zn) greatly exceeded the rat and primate requirements in both what was offered and consumed. The amount of magnesium (Mg) offered was sufficient, but it was not consumed in adequate amounts to meet requirements. Both the amount of iron (Fe) offered and consumed met the requirements for the rat but not for the primate.

Table 5-2. A comparison of nutrient levels offered and consumed by wild *P. tonganus* to values for standard diets of rats and primates..

	Ca	P	Mg	Zn	Fe	Mn	Cu
Rat [1]	0.56	0.33	0.06	11	39	11	6
Primate[2]	0.54	0.43	0.16	11	196	30	10
Diet offered to bats	0.23	0.23	0.21	54	100	9	5
Diet consumed by bats	0.07	0.11	0.07	50	69	4	2
Diet consumed by bats eating leaves*	0.08	0.11	0.08	50	70	5	2

1= NRC 1995, 2= Oftedal and Allen 1996.
*Folivory data are from Chapter 5
Zn, Fe, Mn, and Cu are reported as ppm, and values for Ca, P, and Mg are reported as percent

The addition of minerals due to leaf-eating (Chapter 4) slightly raised ingested mineral levels but overall had little impact on mineral consumption for Tongan fruit bats. Folivory slightly raised Ca and Mg levels, but only to one-seventh of the calcium requirement for rats and one half the magnesium requirement of primates.

Calcium and phosphorus interact and influence the absorption of each other. Calcium is best absorbed when it occurs at a Ca:P ratio of 1:1 to 2:1 (McDowell 1992). In this study, the expected quantity of calcium as well as the Ca:P ratio of 1:1 were not achieved in the food consumed by *P. tonganus*. The diet offered contained a 1:1 ratio, but the Ca:P ratio for ingested food was 0.6:1; approximately half the expected requirement. A low ratio can inhibit calcium absorption making less available to the animal for physiological functions (McDowell 1992).

Additional minerals were available to bats from drinking water. Collected rain water was given daily to bats. The values for 10 ml of Samoan rainwater (n=2 samples) are reported in ppm: Ca = 0.11, P = 0.06, Mg = 0.09, Zn = 0.05, Fe = 0.0, Mn = 0.02, Cu = 0.02. The amount of water consumed by bats remains unknown, so the mineral contribution of water to the diet could not be calculated. Salt licks and calcium blocks may have contributed to mineral ingestion, but use and amount of minerals ingested could not be quantified.

Mineral Absorption

Results of the present study were compared to a parallel investigation on the captive giant flying foxes *P. vampyrus* (Table 5-3). The *P. vampyrus* study was performed on groups of captive bats (Chapter 7) while *P. tonganus* were tested individually, but all other techniques used were identical for both studies. The captive colony of *P. vampyrus* includes lactating females and their offspring as well as non-reproductive females. Mineral apparent absorption values for the 23 *P. tonganus* used in the present study and those of the three pens of *P. vampyrus* have been averaged and presented in Table 5-3 as a single value for each species and mineral.

The apparent absorption values reported for both *P. vampyrus* and *P. tonganus* are similar. *P. vampyrus* met their mineral needs for both amount of minerals offered and consumed for all minerals but copper (Chapter 7). In contrast, *P. tonganus* was deficient in amounts consumed for Ca, P, Mn, and Cu (Table 5-2). There was a difference ($p < 0.05$) in apparent absorption values between *P. tonganus* and *P. vampyrus* for calcium ($p = 0.04$), phosphorus ($p = 0.001$), zinc ($p = 0.001$) and iron ($p = 0.001$), where *P. tonganus* had higher absorption values for those minerals.

Table 5-3. A comparison of mineral apparent absorption values for two species of flying foxes, *Pteropus tonganus* and *Pteropus vampyrus**.

	Pteropus vampyrus	*Pteropus tonganus*
Calcium**	65%	79%
Phosphorus**	63%	84%
Magnesium	68%	73%
Zinc**	39%	85%
Iron**	63%	76%
Manganese	69%	68%
Copper	72%	75%

*Data sets used identical analytical procedures. *P. vampyrus* information is found in Chapter 7.
** significantly different ($p < 0.05$) absorption values between species

Discussion

This study showed that wild Tongan fruit bats have high mineral absorption values. The absorption values for *P. tonganus* are more similar to those found for *P. vampyrus,* another flying fox species, than to either rats or primates (see Table 5.4). However, when compared to *P. vampyrus,* the Tongan fruit bat had significantly higher absorption rates for several important minerals. This suggests that the Tongan fruit bat population may be highly mineral stressed for several macrominerals that are only available in minimal quantities in their diet.

Because the apparent absorption technique has never been attempted before on wild Tongan fruit bats, it is a challenge to find values to compare to those found in the present study. Apparent absorption values for monogastric animals and another species of bat, *Desmodus rotundus*, are presented in Table 5-4 to serve as a basis of comparison to values found for *P. tonganus* in the present study. The values presented are for typical animals that are not under conditions of mineral stress. For the minerals being studied,

values for monogastric animals are typically low and do not exceed 50% absorption under normal conditions.

Table 5-4. Mineral apparent absorption values for selected monogastrics species. Absorption values found in this table are from non-mineral stressed individuals.

Mineral	Animal	Absorption (%)	Reference
Calcium	Human	21%	Coudray et al. 1997
	Fox squirrel	30-39%	Havera 1978
	Vampire bat	16-24%	Coen 2002
Phosphorus	Human	24-31%	Hevesy 1948
	Pig	17-47%	Jungbloed and Kemme 1990
	Vampire bat	18-44%	Coen 2002
Magnesium	Human	46%	Coudray et al. 1997
	Pig	50-60%	Miller 1980
	Vampire bat	25-29%	Coen 2002
Zinc	Human	14%	Coudray et al. 1997
	Rat	17-20%	Tidehag et al. 1988
Manganese	Human	3-4%	Hurley and Keen 1987
	Rat	3-4%	Greenberg et al. 1943
Iron	Human	22%	Coudray et al. 1997
	Rat	9-60%	Fairweather-Tait and Wright 1991
	Vampire bat	5-14%	Coen 2002
Copper	Human	25-70%	Strickland et al. 1972
	Sheep	3-13%	Suttle 1991

However, when dietary minerals are deficient in the diet, mineral absorption often increases due to homeostatic mechanisms that compensate for inadequate intake of that nutrient (Ammerman 1995). Values for normal, non-stressed animals are given as a reference for each mineral. Absorption values for mineral deficient animals are typically 3-4 times higher than are found for animals with adequate mineral intake.

Together, these two tables illustrate how elevated the apparent absorption values are for *P. tonganus* in this study. These values for *P. tonganus* are much higher than are typical of other monogastric animals under normal conditions, and are instead similar tothose found for animals that are nutritionally stressed for minerals. Mineral apparent

Table 5-5. Apparent absorption values for minerals in animals under nutritional stress compared to animals at normal mineral intake levels.

Mineral	Animal	Absorption (%)	Mineral Status	Reference
Calcium	Human	28%	normal intake	Brine and Johnson 1955
		43%	deficient intake	Brine and Johnson 1955
		58%	preterm infant	Bronner et al. 1992
		75%	growing child	RDA 1989
Phosphorous	Human	24-31%	adult	Hevesy 1948
		71%	preterm infant	Koo and Tsang 1991
Magnesium	Rat	26%	normal intake	Brink et al.1992
		57%	deficient intake	Brink et al. 1992
Iron	Human	2-15%	normal adult	Josephs 1958
		20-60%	anemic adult	Josephs 1958
Manganese	Rat	3-4%	adult	Greenberg et al. 1943
		20%	young	Keen et al. 1986

absorption values for *P. tonganus* are most like those of *P. vampyrus*, but *P. tonganus* values are still significantly higher for several minerals. However, the majority of absorption values for *P. vampyrus* are those of lactating females and their pups. The highly elevated apparent absorption values for *P. tonganus* suggest that this population may be highly stressed for mineral nutrients while consuming its current diet in the wild. The diet of *P. tonganus* typically includes a large volume of low-nutrient agricultural fruits (Banack 1996), which potentially contributes to nutrient deficiency in this species.

Bioavailability and Absorption

Bioavailability is defined as the degree to which an ingested nutrient is absorbed in a form that can be used for metabolic functions by an animal (Ammerman 1995). Total intake of a nutrient depends on both the intake and bioavailability of the nutrient (Oftedal 1991). The absorption level of a mineral provides an estimate of its bioavailability. For example, high absorption indicates that the mineral's bioavailability is high and low absorption indicates that the mineral's bioavailability is low. Minerals found in plants are often less bioavailable than in animal sources because fiber in plants binds to minerals

and makes them unavailable for absorption (Soares 1995). Because of this, only 30-50% of ingested calcium in humans is absorbed by the body (Arnaud and Sanchez 1996, Bronner 1998).

Fruit bats consume foods by chewing them into a bolus, swallowing the liquid portion, and ejecting the flattened fibrous pellet (Lowry 1989, Funakoshi et al. 1993, Kunz and Ingalls 1994, Kunz and Diaz 1995). By rejecting the fibrous portion and swallowing the juice, fruit bats may be increasing the bioavailability of the minerals in fruits. Levels of minerals were usually more concentrated in the ejecta pellet than in the diet samples in this study. This suggests that the minerals are in a less soluble form than in fruit and remain in the fiber portion after it has been ejected. To test this hypothesis, apparent absorption was recalculated by including the fibrous pellet in the feces calculation, assuming the portion of calcium in the pellet would not have been absorbed in the gut. The calculation resulted in a 5-20% decrease in mineral apparent absorption values. Thus, the unique pattern of bat consumption may remove the highly absorbable minerals from the pellet by placing them in solution. Because calcium must be in solution to be absorbed (Bronner and Pansu 1999), apparent absorption will be high after ingesting the fruit juices because the ingested minerals are highly bioavailable (Pansu et al. 1993, Duflos et al. 1995,). This manner of feeding results in minerals that are readily absorbed, resulting in high absorption values. Bioavailability and solubility of minerals are crucial under conditions of low mineral intake (Bronner and Pansu 1999).

In addition to their unique feeding behavior, fruit bats have several anatomical adaptations that further increase mineral absorption. The stomachs of fruit bats are large, and the small intestine is long and convoluted, both potential adaptations to increase

absorptive surface area (Dempsey 1999). In addition to the solubility of the mineral, time spent in the intestine is the differentiating factor as to how much mineral is absorbed (Bronner 1998). The relatively long intestine in Old World fruit bats may be up to nine times their body length (Okon 1977). The added surface area of the stomach and intestines may be essential to counteract short gut retention times among fruit bats (30 minutes, Tedman and Hall 1985), and to increase nutrient absorption time of nutritionally-poor foods.

Mineral Stress

Fruit bats may have higher mineral absorption values than other monogastric animals due to their unique feeding patterns and the potentially high bioavailability of minerals in their food. However, high absorption values also suggest mineral stress. An animal will absorb more of a nutrient if the nutrient is deficient in the body or diet (Ammerman 1995). *P. tonganus* had higher absorption levels for many critical nutrients when compared to *P. vampyrus*, a flying fox twice its size (Kunz and Jones 2000). The highly elevated absorption values of *P. tonganus* are of particular interest because *P. tonganus* did not meet its expected mineral requirements for either offered or consumed food, whereas *P. vampyrus* met all of its expected nutrient requirements.

Although agricultural fruits were nutrient poor, and Tongan fruit bats did not meet their expected mineral requirements, bats consistently rejected 41% the offered food and consumed only 85% of their body mass in food nightly. Unlike other bats that consume agricultural fruits, *P. tonganus* did not consume 2.5 times their body mass to meet its nutrient requirements (Dempsey 1999). Although low in nutrients, foods offered to *P. tonganus* were documented as preferred bat foods in American Samoa (Banack 1996, 1998), and were highly preferred to native fruits by fruit bats in fruit preference

experiments (Chapter 3). In addition to being low in nutrients, the foods ingested resulted in a low Ca:P ratio of less than 1:1, which further inhibited mineral absorption (McDowell 1992). Tongan fruit bats consistently chose and consumed low-nutrient fruits that did not meet the mineral requirements of the rat, primate or mammalian standard.

Tongan fruit bats in this study consumed only one-eighth of the expected requirements for calcium and one-third of the phosphorus requirements required for a rat. Yet despite not meeting these requirements, *P. tonganus* births have been observed year round in Samoa, with high numbers of pregnant and lactating females seen throughout the year (Banack 1996). The population has increased three-fold in the last decade following a series of hurricanes that had severely reduced the population (Craig et al. 1994b, Pierson et al.1996, Brooke 1998). Thus, rapid population expansion coupled with the high nutrient cost of bearing a single young (Barclay 1994, Kunz and Stern 1995), and despite the consumption of nutrient-poor food, brings into question the source of minerals, particularly calcium, to support rapid population growth.

The priority of all mammals is to maintain calcium concentrations in plasma close to 2.5 mmol (100 mg) l^{-1} despite fluctuations in the amount of calcium ingested (Arnaud and Sanchez 1996, Hurwitz 1996). This concentration is needed to maintain calcium functions such as cellular metabolism, blood clotting, enzyme activation, and neuromuscular action (McDowell 1992, Soares 1995). Plasma levels will not reflect mineral deficiency for minerals such as calcium, because homeostatic mechanisms maintain calcium levels despite dietary deficiencies (McDowell 1992). Bone acts as a large storehouse for calcium: 99% of the calcium in the body is stored in bone. If plasma calcium concentrations begin to decrease, calcium is quickly mobilized and resorbed

from bone to return plasma calcium levels back to normal (Bronner 1992, Garel 1987). Bone calcium is in a constant state of flux, and it is resorption rather than accretion that is highly responsive in restoring plasma calcium levels (Kwiecinski et al. 1987a).

Changes in bone calcium therefore reflect the extent to which dietary calcium meets the calcium requirements of bats (Bernard and Davison 1996). Females readily allocate their own skeletal calcium to build the skeletons of their offspring, and exhibit marked bone-thinning and structural changes to bone as a result of the calcium demands of pregnancy and lactation (Kwiecinski et al. 1987b, DeSantiago et al. 1999). Calcium levels and bone density can be restored following lactation, with the consumption of an adequate calcium diet (Kwiecinski et al.1987b). However, Tongan fruit bat females in my feeding trials were not consuming a nutritionally adequate diet based on expected values. Their diet was marginal for most of the macronutrients examined in this study, and extremely poor in calcium. Thus, if and how Tongan fruit bats are able to rebuild their skeletons following pregnancy and lactation remains unknown.

Future research

The only true measure of female calcium levels is a bone density test. Porosity of bone serves as an indicator of the calcium status and could determine if the skeletons of *P. tonganus* females are porous and osteoporitic, or are healthy, having recovered from the demands of raising young. To test these ideas further, I predict the following concerning bone density.

1. Flying fox species that prefer agricultural fruits should have lower bone density than those that prefer nutrient-dense native forest fruits. This prediction could be tested using *P. tonganus* and its congener *Pteropus samoensis* in American Samoa (Banack 1996, Pierson and Rainey 1992, Nelson et al. 2000a) or *Pteropus vampyrus lanensis* and *Acerodon jubatus* in the Phillipines (Steir and Mildenstein 2001, Mildenstein 2002).

2. Tongan fruit bat's preference for nutrient-poor agricultural fruits is relatively recent and corresponds with human settlement of the South Pacific islands. This could be tested by conducting bone porosity tests on archaeological bat remains that predate human settlement of the islands and comparing them to the bones of modern Tongan fruit bats (see Steadman 1991, Kirch et. al. 1992).

3. Female bats that consume agricultural fruits and have had many pups should have lower bone density and higher bone porosity than adult non-parous females or adult males of the same species that consume an agricultural diet.

4. Supplementing the diet with calcium should lower mineral absorption rates and increase bone density. The diet of *P. vampyrus* in captivity was supplemented with additional Ca. Therefore, with Ca supplementation, *P. tonganus* should increase bone density and decrease mineral absorption over time, resulting in values similar to those of *P. vampyrus*.

Theoretically, animals should evolve feeding behaviors that enhance the intake of limiting nutrients (Oftedal 1991). Fruit bats may have adapted behaviorally and anatomically to increasing the bioavailability and absorption of minerals from their diet. These adaptations probably arose in response to the evolutionary constraints on increased wing loading from fiber in fruits (Dudley and Vermeij 1992,1994) and to flying fox's preference for high-sugar, low-nutrient fruits (Parry-Jones and Augee 1991a, Nelson et al. 2000a). However, it is not yet known if the preference for high-sugar, low nutrient foods predates the arrival of humans and agriculture on South Pacific islands.

In conclusion, by producing a rejected fiber pellet and swallowing only the juice while eating fruits and leaves, bats may place highly bioavailable minerals in solution. These minerals can then be readily absorbed in the abundant surface area of a large and highly convoluted stomach and small intestine. The elevated apparent absorption values found in *P. tonganus* suggest that ingested minerals are either highly bioavailable and readily absorbed, or this population is in severe mineral stress due to its preference for agricultural fruits. Future work may distinguish if elevated absorption values are unique to Tongan fruit bats or are typical of other flying fox populations. Much work remains to

be done on the mineral nutrition of fruit bats so that base values can be established for different species and animals in different physiological states.

CHAPTER 6
NUTRITIONAL LANDSCAPE ECOLOGY AND HABITAT USE BY TONGAN FLYING FOXES IN AMERICAN SAMOA

Introduction

Flying foxes of the genus *Pteropus* are strong fliers capable of traveling long distances (Nelson 1965, Eby 1991, Spencer et al. 1991, Banack 1996, Palmer and Woinarski 1999, Shilton et al. 1999, Palmer et al. 2000). *Pteropus* species may commute up to 50 km nightly, traveling at speeds of 40 km/h while searching and foraging among food patches throughout the landscape (Richards 1990, Spencer et al. 1991, Palmer and Woinarski 1999, Banack 1996). This allows flying foxes to access patchily distributed fruit, nectar, and flowers, and to avoid local food shortages by seeking out distant, scattered food resources (Bronstein 1995). Thus, an entire forest or all of a small oceanic island may represent potential foraging habitat to a flying fox.

The selection of a foraging patch within a heterogeneous landscape has been described as a hierarchical decision process that occurs at different levels: regional, landscape, plant community, or at the level of the individual plant (Senft et al. 1987). Potentially using significant powers of spatial memory and learning, flying foxes range widely with information on both the location and quantity of resource patches within the landscape (see Lima and Zollner 1996, Zollner and Lima 1999). For example, the black flying fox (*Pteropus alecto*) exploited landscape patchiness at two scales, between broad vegetation types and within vegetation types, and selected sites that were rich in resources from among the homogeneous forest matrix (Palmer et al. 2000). Its selection

highlighted the patchiness of resources in the landscape (Palmer et al. 2000) and demonstrated the bat's awareness of differences in habitat quality among the patches.

Animals should utilize resource patches within the landscape in a way that maximizes fitness (Lima and Zollner 1996). In certain stages of a life cycle, nutritional needs can be very specific, and filling those needs can be crucial for population survival. Habitats in which such needs can be met are often considered key to population persistence (Kozakiewicz 1995). For example, calcium is often limited in the diet of bats, and females may be calcium stressed during pregnancy and lactation (Keeler and Studier 1992, Radostits et al. 1994, Studier et al. 1994a, Bernard and Davison 1996). Inadequate levels of dietary calcium can result in low milk production in females and inhibit growth in offspring (McDowell 1992, Radostits et al. 1994). Limited calcium availability in the diet has the potential to limit fecundity and survivorship of animal young (Batzli 1986), which may affect population levels in bats (Barclay 1995). Increased calcium requirements during pregnancy and lactation may be relieved by the consumption of concentrated sources of calcium such as figs (*Ficus* spp.) or other calcium-rich fruits (Nelson et al. 2000a, Ruby et al. 2000). Foraging areas that contain concentrated sources of calcium should potentially be important for reproductive bat populations and for population persistence.

Before the arrival of Polynesians more than 3,000 years ago, most of Tutuila, American Samoa, was covered in native rainforest (Cole et al.1988, Whistler 1992, Hunt and Kirch 1997). Since then, human activities such as land clearing and shifting cultivation have altered much of the forest area and replaced rainforest with mixed crops and residential areas (Cole et al. 1988). Previous nutritional analysis of native and

agricultural fruits indicate that native fruits are a much more concentrated source of nutrients, particularly for the mineral calcium (Nelson et al. 2000a). Thus, patches that contain native fruits may represent higher quality habitat than those that contain nutrient-poor agricultural fruits. Habitat patches that contain calcium-rich fruits could potentially be used to increase dietary calcium consumption.

The Tongan fruit bat, *P. tonganus*, is a habitat and feeding generalist, that forages in both native and agricultural areas on 42 species of plants on Tutuila, American Samoa (Wilson and Engbring 1992, Trail 1994, Banack 1996), but the extent of use of each habitat type is unknown (Brooke 1998). Tongan flying foxes are able to transverse the length of Tutuila island in a single night, and use different sides of the island for foraging throughout the night (Banack 1996). Tutuila is isolated from other islands by an ocean barrier of 100 km. Thus, the island represents a single, isolated, heterogeneous foraging area. This results in a unique situation that allows one to study the entire foraging area available to *P. tonganus* in American Samoa.

Nutritional landscape ecology combines the concepts of nutritional ecology and landscape ecology to determine if animals select nutrient-rich areas as they forage within the landscape. In the present study, I evaluated whether Tongan fruit bats foraged preferentially in high calcium habitat types to increase consumption of calcium. It was predicted that Tongan flying foxes, particularly pregnant and/or lactating females, would forage within calcium-rich areas to obtain supplemental calcium in their diet.

Methods

Major Vegetation Types

This study was conducted between February 2000 and August 2001 on Tutuila, the largest island in American Samoa (14° S, 170° W) in the South Pacific Ocean. The

three major vegetation types on present-day Tutuila are native forest, mixed agroforest, and village agricultural areas. Native forest is the climax forest for the island and includes upland, mangrove, moss, and coastal forest (Cole et al. 1988, Whistler 1994). Native forest on Tutuila includes a rich diversity of plant and tree species with a 30% level of endemism (Whistler 1992, 1994). Mixed agroforest results from disturbance, either natural or anthropogenic, and is a transitional stage between plantation land and native forest. In this forest type, fruit trees are planted among secondary growth forest trees (Cole et al. 1988). Village agricultural lands include land cleared to grow fruit trees in sparse density, and are adjacent to residential areas that include villages, plantations, and roads (Cole et al. 1988). A vegetation habitat map of Tutuila was available for Tutuila and coded for the three major vegetation types (Cole et al. 1988, Freifeld 1998).

Nutritional Classification of the Major Vegetation Types

The three vegetation types of the island were categorized as either high, intermediate, or low in nutrient availability based on the calcium content of the fruits found within each habitat (Table 6-1). Nutritional analysis indicated that minerals were more concentrated in native fruits than in agricultural fruits (Nelson et al. 2000a). Figs are an especially rich source of calcium and are found mostly in native forests (Whistler 1992, Nelson et al. 2000a). Because native forests contain calcium-rich fruits and figs, these forests represented calcium-rich habitat. Mixed agroforest represents intermediate-calcium habitat because it contained both native and agricultural fruits. Village agricultural land was considered calcium-poor habitat because it contained only agricultural fruits that were nutrient-poor and sparsely distributed in the landscape.

Table 6-1 Nutrient classification of habitat types in American Samoa.

Habitat type	Sample of trees used by fruit bats*	Calcium content (mg/g)**	Habitat classification
Native forest	*Ficus scabra*	10.30	Calcium-rich
	Planchonella garberi	4.66	
Mixed agroforest	*Carica papaya*	2.46	Intermediate calcium
	Myrstica fatua	n/a	
Village agriculture	*Musa spp.*	0.55	Calcium-poor
	Artocarpus altilus	0.91	

* from Whistler 1992, 1994, Cole et al. 1988, Banack 1996.
** from Nelson et al. 2000a

Netting of Bats

Bats were captured using mist nets set at several foraging sites throughout the island. Sites were chosen by watching animal movements at dusk, by finding fruit ejecta pellet locations, and on the advice of local residents. Bats were captured in large mist nets (6-18 m, 4 inch mesh, Avinet, Inc., Dryden NY) attached to pulleys placed high in coconut trees (approx. 15 m) or on tall poles. Nets were raised at sunset and were checked every 30 minutes until midnight when they were taken down. Netting was terminated in the event of rain, a full moon, or excessive wind. Bats were caught from the center, east, and west side of the island so that results represent the entire island population. After capturing a bat, its sex, reproductive status, overall body condition, forearm length, body mass, and time of capture were recorded (Racey 1988).

Radiotelemetry

Radiocollars (Model RI-2D, Holohil Systems, Ltd) were fitted around the necks of bats using embroidery thread strung inside Tygon rubber tubing (2 mm). The transmitters weighed 8.5 g and represented 3% of the bat's body weight. Collars often fell off or were removed by bats but were retained for an average of 2.5 months. They were recovered, refurbished, and used again. Nocturnal radiotracking involved tracking bats on

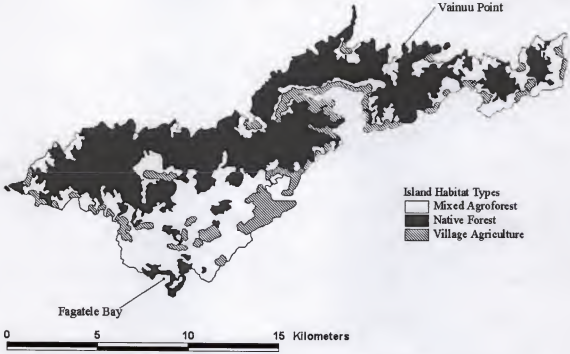

Figure 6-1. Map of Tutuila, American Samoa showing the three island habitat types. Map follows Freifeld 1998.

foot or from a truck along roads and trails. The loudest signal method (Springer 1979, Kenward 2001) was used to determine the direction to the signal. By sighting down the mast of the antennae with a compass, an azimuth was taken. Three azimuths whose intersecting angles were generally 30° but no less than 20° apart were used to estimate the bat's location. The program Locate (version 2.82, Nams 2001) was used to generate the location estimates.

Bats were tracked two to three nights a week and only once per night to maintain independence of locations (Erickson et al. 2001, Kenward 2001). Locations were collected in the first six hours of the night (18:00-24:00 h), beginning shortly after bats left the roost to forage and continuing until midnight. Collection terminated at midnight because bats displayed a sharp decrease in activity beginning at midnight and continuing until early morning (Banack 1996). The sequence in which individual bats were located

was random and opportunistic. Locations taken early in the night were preferred to test if calcium was sought first, although previous radio-tracking work indicated that *P. tonganus* often used a single foraging area throughout the night (Banack 1996).

A digital USGS 7.5" map of Tutuila was proportioned and georeferenced by using ESRI's Image Analyst and 18 GPS locations taken across the island. The Freifeld vegetation types map (Freifeld 1998) was overlaid onto the USGS map and proportioned using Image Analyst. The map image was then converted to an ArcView shape file for use in habitat analysis. The map was ground-verified at 175 points to check for accuracy of the resulting map. The three areas that received the most bat use were mapped with a GPS, converted to GIS, and used for the habitat analysis. Habitat use was determined by the number of times each bat was located within each habitat type. The Chi-square goodness of fit test and simultaneous Bonferroni confidence intervals (Neu et al. 1974) was used to determine if habitat types were used in proportion to their availability (see Erickson et al. 2001). Tests of habitat selection were performed for three groups; males, reproductive females, and non-reproductive females. To evaluate differences in foraging distances flown by bats, SPSS (Norusis 1993) was used. A Kolmogorov-Smirnov test revealed that the data were not normally distributed, so the non-parametric Mann-Whitney test was used to analyze the data (Sokal and Rohlf 1995).

Radiotelemetry Error

Data obtained by two independent observers were evaluated for telemetry error using the Location Error method (Zimmerman and Powell 1995) and error ellipse results from the Locate program. Fourteen test collars were placed in different habitat types and at different distances around the study area to account for the different effects each habitat type and distance may have on the radio signal. True collar locations were

determined using a handheld GPS (GeoExplorer 2, Trimble Navigation, Ltd., Sunnyvale, CA). Error was assessed for both observers independently taking radiotelemetry locations. A two-sample t-test (Sokal and Rohlf 1995) was performed on the results of 27 collar locations to evaluate the potential difference in performance between the individuals radio-locating bats. The data were transformed by natural log to approximate a normal distribution.

Results

Radiotelemetry Error

The results of the t-test showed no difference ($p = 0.27$) in the error distances between the two observers, thus the results were pooled to produce an overall study error distance for all radio-location estimates. The mean error for the straight-line distance between a known location and a location estimate was 103 ± 138 m. For comparison, the mean error ellipse from the Locate location estimate was 1.72 ± 2.63 ha with a median of 0.54 ha. The standard deviation of 2.63 ha is less than a 100 m straight-line distance.

Habitat Selection

Radiocollars were attached to twenty (11 males, 9 females) Tongan flying foxes. Two of the nine females were lactating and thus classified as reproductive. All other females were considered non-reproductive. Seven months of radiotelemetry resulted in 166 usable locations. Bats left the roost to begin foraging at approximately 18:00. Radiotelemetry locations were taken between 18:00 and 24:00 h. (Figure 6-2)

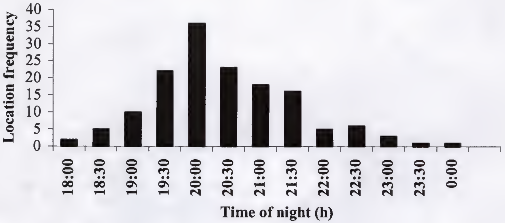

Figure 6-2. Frequency of *P. tonganus* location estimates recorded from 18:00 to 0:00 on Tutuila, American Samoa.

Mixed agroforest provided 70% of all radiotelemetry locations (116/166 locations) and was preferred ($p < 0.001$) over the native forest or village agricultural habitat types (Table 6-2). Mixed agroforest was used by both sexes and all ages of bats across a wide spectrum of time. Native forest was avoided by bats ($p = 0.05$) and was used only 20% of the time. Village agriculture was used only by non-reproductive females (n= 9 locations), and never by males or reproductive females. Reproductive females (n=2) were located only within mixed agricultural areas. A broken collar on one of the females prevented us from getting a greater number of locations for reproductive females (n = 10). Eight dropped collars were found; five in mixed agricultural areas, two in village agricultural areas, and one in native forest habitat over the seven months.

Table 6-2. Summary of goodness-of-fit tests for habitat selection for radio-collared Tongan flying foxes on Tutuila, American Samoa.

	n	X^2	DF	Habitat type[a]		
				Village agriculture	Mixed agroforest	Native forest
All bats	20	117.18*	19	NS	Preferred	Avoided
Males	11	69.43*	10	Avoided	Preferred	Avoided
Non-reproductive females	7	41.69*	6	Preferred	NS	Avoided
Reproductive females	2	87.70*	1	Avoided	Preferred	Avoided

* significant at $p < 0.001$, indicating that habitats were not used in proportion to availability.

[a] Avoid = habitat used less than expected based on its availability
Prefer = habitat used more than expected based on its availability
NS= no selection, habitat used in proportion to its availability

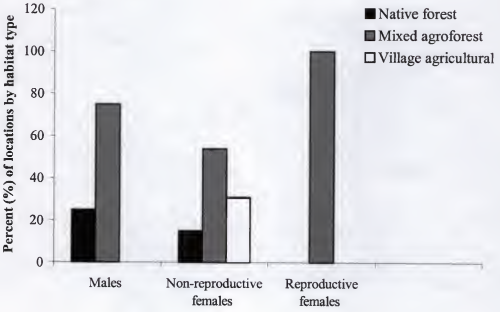

Figure 6-3. Percent of locations by habitat type on Tutuila, American Samoa used by Tongan flying fox males (n= 11), non-reproductive females (n = 7), and reproductive females (n=2).

Distance Traveled from Roost to Foraging Site

All bats used in this study either roosted on the southwest side of the island at Fagatele Bay on Mataautuloa Ridge, or on the northeast side of the island near Afono Village at Vainuu Point on Ogetu Ridge (Figure 6-1). Each bat was tracked for an average of 2.5 months before the collar fell off or we were unable to locate the bat on the island (Table 6-3). The average flight distance for bats from roost to foraging site was 1.8 km. Males and females did not differ in the distance flown from roost to foraging site (1.5 km males, 2.3 km females). Both reproductive females were identical in their average foraging distance (0.7 km). Although their average distance flown was much less than that of other females (0.7 km vs 2.3 km, respectively), the results were not significantly different ($p = 0.55$), most likely due to the small sample size of lactating females ($n=2$).

Bats from roosts on the west side of the island (at Fagatele Bay, $n= 15$) flew an average of 0.87 km to their feeding locations, while bats on the east side (at Vainuu Point, $n=5$) flew an average of 4.84 km (Figure 6-4). These foraging distances were significantly different ($p = 0.002$). The single longest straight-line distance flown by a bat from the east-side roost (Vainuu Point) was 16km by an adult female. The longest straight-line distance flown from a bat from the west-side roost (Fagatele Bay) was 8.1 km by a young adult female.

Table 6-3. Data summary for the 20 Tongan radio collared fruit bats in American Samoa.

Individual	Sex	Age	Number of months tracked	Number of radiotelemetry fixes	Ave. roost to foraging distance(km)	Roost location on Tutuila
1	M	Subadult	2	10	0.9	West
2	F	Subadult	2	3	0.6	West
3	M	Subadult	2	11	0.4	West
4	F	Adult	1	7	0.7	West
5	F	Adult	4	10	2.4	East
6	M	Subadult	2	6	0.9	West
7	F	Subadult	2	4	0.6	West
8	M	Subadult	2	8	0.7	West
9	M	Adult	2	6	0.7	West
10	M	Subadult	2	5	0.7	West
11	M	Subadult	4	14	7.2	East
12	F	Subadult	3	14	3.2	West
13	F	Adult	2	5	2.8	East
14	F	Adult	3	5	9.0	East
15	M	Subadult	2	10	2.8	East
16	M	Subadult	1	7	0.9	West
17	M	Adult	4	21	0.7	West
18	F	Adult	3	19	0.7	West
19	M	Adult	2	14	0.6	West
20	F	Adult	1	3	0.7	West

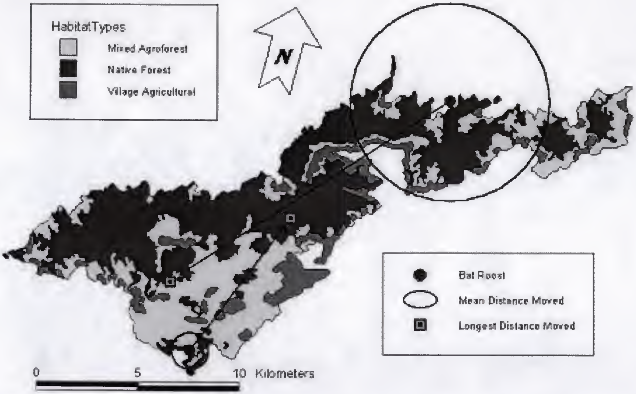

Figure 6-4. A map of Tutuila, American Samoa showing the mean distance flown from each roost site for *P. tonganus* and the longest distance moved by a bat from each roost.

Discussion

Radio-tracking studies can highlight what habitat types are preferred by flying foxes, and if reproductive females prefer nutrient rich areas for foraging. In this study, Tongan flying foxes preferred the mixed agroforest habitat type to all other habitat types. Mixed agroforest represented 70% of all radiotelemetry locations. Non-reproductive females were the only group that preferred to forage in village agricultural areas, and reproductive females foraged only within mixed agricultural areas. Bats roosting on the east side of the island flew farther to forage than bats roosting on the west side of the island. Reproductive females flew less distance than non-reproductive females to forage.

Habitat Preference

The observed preference that *P. tonganus* displayed for mixed agroforest habitat in this study is consistent with other studies on this species (Pierson et al. 1992, Wilson and Engbring 1992, Banack 1996, Brooke 1998). The results of this landscape-level study are also in agreement with the results of fruit preference tests at the level of the individual bat, where agricultural fruits were overwhelmingly preferred to native fruits in feeding trials (Chapter 3). Dropped collar locations further support a preference for the agroforest habitat type and may be a reflection of time spent foraging in that habitat type.

Tongan flying foxes exhibited variation in habitat use among males and females. Non-reproductive females were the only individuals to use village agricultural areas for foraging. Reproductive (lactating) females only fed in mixed agroforest areas. In contrast, males were never located in village agricultural areas. In Australia, Spencer and Fleming (1989) also found that females (*Nyctimene robinsoni*) were more likely to feed on fruit in fruit orchards than males, whereas males preferred to feed on native figs. Because figs are calcium rich (O'Brien et al. 1998, Nelson et al. 2000a), and reproductive or parous

females may be calcium deficient, it seems counterintuitive for males, but not females, to prefer them. Elangovan et al (2001) found that carbohydrates and water were consumed first following the extended day roosting period. Females may be more water or energy stressed than males, particularly females while lactating. Agricultural fruits tend to be both juicier and more sugar-rich than native fruits (Oftedal and Allen 1996). This may contribute to a preference for agricultural fruits, particularly among reproductive females that emerge following a period of fasting in their day roost and demands associated with lactation.

Distance Flown from Roost to Foraging Site

Flying fox species typically fly 10-30 km between roost sites and feeding locations (Mickleburgh et al. 1992, Palmer and Woinarski 1999). Previous research in American Samoa indicates that Tongan flying foxes are capable of flying 45 km in a single night (Banack 1996). While a single adult female in this study flew 16 km from a roost to a foraging location, this behavior was not typical of the group. Instead, Tongan flying foxes in this study averaged less than 2 km flying distance between their roost and foraging locations. Bats flew approximately 1/10 the distance reported for *P. tonganus* in 1992-1994 on the same island (Banack 1996).

Differences in the distances flown by *P. tonganus* may be related to the years in which the studies were done. Banack's study was conducted two years after a series of destructive hurricanes battered the Samoan archipelago. With sustained winds in excess of 200 km/hr, the hurricanes stripped trees of their fruit and leaves, and severely reduced the food base of the island (Elmqvist et al. 1994, Pierson et al. 1996). For several years following the hurricanes, food was scarce on Tutuila, and Tongan flying foxes had to fly

farther to find food (see Craig et al. 1994b, Nelson et al. 2000b). In the decade since the hurricanes, food has become plentiful on the island. Monthly fruit surveys conducted in 2001 demonstrated that agricultural fruits were abundant year round on Tutuila (S. Nelson, unpublished data, Trail 1994). The year-round abundance and availability of agricultural fruits compared to native fruits may have resulted in both a preference for them and shorter foraging distances from the roost to find them. Foraging distances have been shown to decrease in other bat species when resources were plentiful and increase when resources were scarce (Spencer and Fleming 1989, Palmer and Woinarski 1999).

Foraging Distance and Roost Affiliation

The greatest differences in foraging distances among *P. tonganus* were related to roost affiliation. Bats that roosted on the east-side of the island traveled an average of 5.5 km farther to forage than did the bats that roosted on the west side. Both roosts were in native habitat, as is typical of this species (Brooke 1998). However, the roost on the west side of the island (at Fagatele Bay) was near a large agricultural area. In contrast, bats on the east side of the island were near the National Park of American Samoa, which is primarily native forest habitat (Cole et al. 1988, Whistler 1994). Although native forest provided a nutritionally richer resource, bats from the east side flew across the island nightly to feed in mixed agroforest areas. For example, an adult female flew an average of 9 km each night from her east-side roost to feed within different mixed agroforest areas on the west side. Other flying foxes that roost in native forests but feed in agricultural areas also fly long distances to reach the agricultural areas (Mildenstein 2002).

Foraging Patterns of Reproductive Female Bats

Reproductive females exhibited atypical foraging behaviors when compared to non-reproductive female bats. The two lactating females traveled less than any other group of bats. Both lactating females roosted on the east side of the island (Fagatele Bay) but flew less than the average foraging distance for other bats that also occupied that roost. These two females also flew identical distances (0.7 km) and flew the same distance each night they were tracked to foraging areas (n=10). Foraging distances for reproductive females may be restricted to areas near the maternity roost because they have dependent young (Palmer and Woinarski 1999). This may explain the consistency in their flight distances and the relatively short distances flown by both reproductive females. Dominque (1991) found that pregnant and lactating *Carollia perspicillata* females flew almost as many flying bouts as non-reproductive females, but their flights were much shorter. In contrast, non-reproductive females performed longer exploratory flights to survey for fruit abundance. Thus, the feeding behavior seen in reproductive females may be a means of shifting energy from exploratory behavior to reproductive effort (Dominique 1991).

Nutritional Landscape Ecology

Little is known about the kind of information available to animals at the scale of ecological landscapes, and how this information is used with respect to habitat selection (Lima and Zollner 1996). The central prediction of this study was that reproductive females would forage in calcium-rich habitats to relieve calcium deficiencies that potentially arise during pregnancy and lactation (Keeler and Studier 1992, Radostits et al. 1994, Studier et al. 1994a, Bernard and Davison 1996). However, a strong preference for calcium-poor agricultural habitat areas and an avoidance of calcium-rich native forest

habitats suggests that Tongan flying foxes may forage to maximize the intake of nutrients other than calcium. Agricultural fruits were selected despite the calcium deficiency that resulted from consuming them, based on an assumption of the standard (Chapter 3,5). While foraging, Tongan flying foxes appeared to seek out fruits that were high in energy-rich carbohydrates (sugar), similar to what was reported for *Cynopterus sphinx* (Elangovan et al. 2001, Chapter 3, 7).

A potential limitation of this study was the bias toward documenting initial foraging flights. Elangovan et al. (2001) found that *C. sphinx* fed on predominantly energy-rich fruits during the early hours of the night, and foraged for concentrated mineral sources later in the night. Sugar-rich agricultural fruits provide the highest energy return for an animal's foraging effort, and may relieve a carbohydrate and water debt incurred while at the day roost (Kurta et al. 1989, Elangovan et al. 2001). Initial foraging flights were prioritized under the assumption that bats would first forage to relieve calcium deficiencies. Early night was also chosen to avoid the period of rest typical of *P. tonganus* later in the night (Banack 1996). In addition, because all lands are private in American Samoa, work was terminated at midnight to avoid disturbing residents sleeping in open houses (fales) while tracking bats on private land. However, Banack (1996) found that most *P. tonganus* foraged within a single area throughout the night, following a period of foraging upon arrival in the area (Banack 1996). In another study, *C. sphinx* left the roost to begin foraging at 18:00, and commenced leaf-eating at 19:30 h (Elangovan et al. 2001). Almost 90% of the radiotelemetry locations in this study were recorded after 19:30. Thus, these radiotelemetry locations may include leaf-eating, and reflect the consumption of high-energy fruits earlier and leaves later in the night.

Due to the paucity of alternative vertebrate pollinators and seed dispersers, fruit bats are considered keystone species on Tutuila (Cox et al. 1991, Banack 1998, Rainey et al. 1995). A decline in bat population size could affect community structure and biodiversity on the island. Mixed agroforests were highly preferred by Tongan fruit bats, and their maintenance is important for successful foraging. *P. tongnanus* and its congener on Tutuila, *Pteropus samoensis*, are both dependent on native forest for roosting (Brooke 1998, 2001). It is critical to preserve native forest on Tutuila to maintain roosting habitat and to create a buffer from anthropomorphic disturbance. Disturbance of maternal roosts can lead to abandonment of the roost and have population-level effects (Brooke 1998).

In summary, Tongan flying foxes showed a strong preference for agricultural habitats. Bats commuted nightly over native forest habitat to feed in mixed agroforest areas or fed in adjacent mixed agroforest areas near their roosts. Tongan flying foxes appeared to forage in a manner that maximized their energy intake rather than their calcium intake. Succulent and sugar-rich agricultural fruits were possibly preferred by hungry and dehydrated bats emerging from the day roost as a source of quick, high-energy food. Agricultural fruits were plentiful and available year-round on Tutuila, which may have resulted in minimum foraging distances to find them. Reproductive females may be constrained to forage near the maternity roost to support energy-demanding lactation and, if so, reproductive females should prefer high-energy, locally abundant agricultural fruits. Future habitat use studies of flying foxes should include radiotelemetry locations taken throughout the night and an investigation of foraging within a larger group of reproductive females. Together, these studies may prove decisive in determining if *P. tonganus* attempts to increase their calcium intake by feeding in nutrient-rich areas.

CHAPTER 7
ABSORPTION AND UTILIZATION OF MINERALS CONSUMED BY CAPTIVE LACTATING FEMALE MALAYAN FLYING FOXES (*PTEROPUS VAMPYRUS*) AND THEIR PUPS

Introduction

Fruit bats of the suborder Megachiroptera and family Pteropodidae are increasingly bred and maintained in captivity. However, little is known about either their mineral requirements or the adequacy of the diets fed to them. Because nutritional standards are unknown, dietary recommendations for fruit bats have been based on standards for rats, averages for all mammals, or diets that maintain breeding colonies of flying foxes in captivity (Fascione 1995, NRC 1995, Dierenfield and Seyjagat 2000b).

Two separate factors, the overfeeding of heterogeneous diets, and dominance hierarchies in social species, are now recognized as important factors influencing the nutritional intake of captive wildlife (Robbins 1993). The distribution and composition of the daily diet given to fruit bats in captivity are quite different than that consumed by free-ranging bats. In the wild, *Pteropus vampyrus* flys up to 50 km each night to reach its feeding grounds, and the temporal and spatial distribution of food resources are complex, so that food acquisition often requires a large proportion of an animal's time budget (Medway 1969, Oftedal and Allen 1996, Kunz and Jones 2000). In addition, wild animals do not eat more than they require because this effects their wing loading and ability to fly (Dudley and Vermeij 1992). In captivity, energy requirements are less because bats do not travel to their food source, and flight is restricted by cage size (Courts and Feistner 2000). Thus, the combination of reduced activity and plentiful food result in captive bats

that are heavier than their wild counterparts. This may lead to obesity in dominant individuals (Allen and Oftedal 1996, LeBlanc 1999).

When captive animals are housed in groups, it is common practice to feed amounts somewhat in excess of consumption to ensure that all individuals have access to food, and that young and subordinate animals receive adequate quantities (Courts and Feistner 2000). Animals often choose among the numerous food items offered, and may ingest a diet that is much different from the diet that was offered. Therefore, assessments of the nutritional adequacy of a diet should be based on what is actually eaten rather than what is offered (Oftedal and Allen 1996). Intake levels also vary with origin, age, dominance, and reproductive status of animals (Courts and Feistner 2000).
To accurately document mineral intake, this study evaluated mineral absorption efficiencies using apparent absorption. This method measured both mineral intake and excretion, and accounted for the unique manner of fruit bat feeding where bats chew the plant matter into a fibrous pellet, swallow the juice, and eject the flattened pellet (Lowry 1989, Kunz and Ingalls 1994, Kunz and Diaz 1995). Previous studies failed to account for minerals found in ejected pellets.

Growth, pregnancy, lactation, age, gender, nutrient interactions and illness can influence nutrient requirements. Appropriate amounts of calcium and phosphorus are especially critical for bats during early growth and peak lactation (Barclay 1995, Hood et al. 2001). If dietary intake of calcium is inadequate, pregnant and/or lactating females donate their own skeletal calcium to build the skeletons of their young (Bernard and Davison 1996). Inadequate dietary calcium can result in weakened bones and low milk production in females (Radostits et al. 1994). Patterns of post-natal growth in the pups are

influenced by the quantity of milk from the female fruit bat (Kunz and Stern 1995, Kunz and Hood 2000). Inadequate calcium results in inhibited growth and reduced mineralization of bone in offspring (McDowell 1992, Radostits et al. 1994). Additionally, nutritional conditions in captivity may push post-natal growth rates to their maximum, adding further nutritional stress (Kunz and Stern 1995, Kunz and Hood 2000). Thus, it is critical that adequate minerals are available in the diet of lactating females and their rapidly growing offspring.

In this study, two pens of lactating female *P. vampyrus* and their rapidly growing pups were compared to a control of non-reproductive *P. vampyrus* females to evaluate differences in dietary intake and assimilation of minerals as determined by apparent absorption. The large size of the Malayan fruit bats, coupled with the nutrient demands of lactation and rapid growth (Barclay 1994, Kunz and Hood 2000), served to illustrate if their diet is nutritionally adequate for a breeding population of large captive flying foxes.

Methods

This study used *Pteropus vampyrus*, also called the Malayan flying fox, to examine mineral nutrition in captive fruit bats. *P. vampyrus* is one of the world's largest flying foxes, weighing approximately 1 kg and attaining a wingspan of over 2 m (Ingle and Heaney 1992, Kunz and Jones 2000). Males generally weigh more than females, and overweight males in captivity can weigh as much as 1.5 kg. Populations of female *P. vampyrus* give birth synchronously during a single annual peak, usually to a single offspring (Mickelburgh et al. 1992). The gestation period is approximately 180 days after which young suckle from their mothers for 2-3 months, but young may depend on their mothers for as long as a year (Pierson and Rainey 1992, Kunz and Jones 2000).

Three pens of *P. vampyrus* were tested within their normal outdoor flight cages at the Lubee Foundation, Inc. in Gainesville, Florida in January 2000. Two pens (pens 1 and 2) consisted of lactating female *P. vampyrus* and their rapidly growing pups. A separate pen (pen 3) served as a control and contained only non-breeding females. Bats were kept in octagonal outdoor flight cages that contained an enclosed indoor portion (the core) where all food was suspended and consumed. Bats were fed their normal diets (Table 7-1) and were fully acclimated to both their surroundings and diet for two months before the experiment began. Bats were fed approximately one-half their body mass per day (wet weight). Stainless steel bowls of food were hung near the top of the core to prevent fecal and urinary contamination. Food was available to the bats from 1500 to 0900 h each day. Water and salt licks were available to the bats at all times. Salt licks were composed of salt and mineral oil and contained 96-99 % salt (Pet Products, Inc., Hauppauge, N.Y.). Water samples contained 0.10 mg calcium, 0.20 mg phosphorus, 0.3 mg magnesium, 0.05 mg potassium, 0.03 mg zinc, 0.20 mg iron, 0.30 mg manganese, and 0.31 mg sodium for every 1 ml of water offered to the bats. Copper levels were not detectable.

Table 7-1. Composition of food fed to *P. vampyrus* at the Lubee Foundation, Inc.

Fruit type	Percent of diet (%)
Apple	35.9
Pear	6.8
Banana	13.6
Juicy fruit (grapes, kiwi, pineapple)	7.8
Mixed fruit (seasonal items)	2.6
Cantaloupe	12.0
Carrot/sweet potato (steamed or raw)	9.1
Kale/spinach	6.5
Dry Zupreem primate biscuit*	5.0
Vitamin E solution	0.1
Dicalcium phosphate	0.6

*this was replaced with Lubee Fruit Bat Supplement in February 2001.

Two days of trials were performed in the three pens. Upon arriving at the indoor enclosures the next morning, rejected food and feces littered the enclosure floor. Food remains were separated into three categories: 1. uneaten food that remained in the bowls and rejected food on the floor (orts), 2. food that had been masticated, the juice swallowed, and the fiber pellet rejected (ejecta), and 3. fecal remains (feces). Samples of orts, ejecta, and feces were collected by scraping the samples off the floor and walls using a sterile plastic paint scraper or picking them up using stainless steel tweezers. Both the scraper and tweezers were rinsed in deionized water between samples. Latex gloves were worn during collection to further prevent contamination. Some of the orts and ejecta samples on the floor of the enclosure were contaminated with feces. Those samples were sprayed with distilled water to remove as much fecal matter as possible. Fresh masses were recorded and subsamples were pooled for each pen and frozen for future analysis. Dehydration factors for all subsamples were subtracted from wet weight values.

Samples were analyzed at the Animal Nutrition Laboratory at the University of Florida in Gainesville, Florida. Frozen samples were thawed and placed in a drying oven for 24 h at 105° C. Dried samples were weighed and ashed at 550°C for 12 h. Each sample was prepared and digested according to the procedures of Miles et al. (2001). Following wet digestion, calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) contents were assessed by flame atomic absorption spectrophotometry using a Perkin-Elmer AAS 5000 (Perkin-Elmer 1980). Phosphorus was measured separately using a colorimetric assay (Harris and Popat 1954). All samples were analyzed in duplicate. Standard reference material (citrus leaves

1572, National Institute of Standards and Technology, Gaithersburg, MD) was analyzed with each sample set for accuracy.

The combination of uneaten food in the bowls and food ejected was subtracted from total food offered to obtain a value for total food intake. The mineral concentration of fruit was multiplied by the amount of food that was consumed each day to calculate total mineral intake. Total mineral intake was used to calculate values for apparent absorption. Both equations are presented below. All calculations are on a dry matter basis. Dry matter numbers may be slightly lower than the actual fruit value for all fruits because the fruit was in the heated core, which may have desiccated the samples.

Total mineral intake (g) =
(total amount of fruit offered (g)) – (total amount of orts (g)) – (total amount of ejecta (g))

Apparent absorption (%) =

$$\frac{\text{(total mineral intake)} - \text{(fecal mineral concentration)}}{\text{(total mineral intake)}} \times 100$$

All values for diet, ejecta, orts, fecal, intake, retention, and apparent absorption were statistically analyzed using the General Linear Model (GLM) procedure in SAS (SAS 1999). This procedure was used to test for a difference in the means among the three pens. Significance was evaluated at the $p < 0.01$ level because of the large number of comparisons tested, and that the values were not all independent measurements, but were based on previous values (e.g., retention and absorption) (R. Littell, pers.comm.).

Results

Nineteen lactating females and sixteen pups were compared to twelve non-breeding female *P. vampyrus* to evaluate dietary differences between the groups (Table 7-2). Three of the females in the lactating pens were without pups due to the death of two pups and one infertile female. The pups were from a single breeding cohort and were all

6-8 months old. *P. vampyrus* pups at the Lubee Foundation, Inc. are usually in the weaning process at four to six months of age, but they will continue to nurse opportunistically until they are eight months old (D. LeBlanc, pers. comm.). Many fruit bat species continue to nurse at one year of age (Galindo et al. 1995, West 1986).

To compensate for the demands of prolonged lactation in females and growth in pups (Radostits et al. 1994), females in pens 1 and 2 were given twice the amount of food (wet mass) that the non-breeding females received in pen 3. The two groups of lactating females and their offspring were very similar in the dry diet consumed as a percent of body mass (11.2% and 10.5% respectively). Non-breeding females consumed approximately 3% less than the lactating females with pups. Lactating females presumably ingested more food to compensate for the energy demands of lactation.

Table 7-2. A comparison of lactating female *P.vampyrus* with pups to nonbreeding females for number of bats in pen, diet offered (wet mass), and dry matter intake as a percentage of body mass. M: male, F: female, N/A, data not available, pups were nursing.

Pen	Treatment group	Number of bats in pen	Diet offered	Dry matter intake as % of body mass
Pen 1	Lactating females	10	11,314g	11.2%
	Number of pups	4M, 5F	N/A	N/A
Pen 2	Lactating females	9	10,565g	10.5%
	Number of pups	4M, 3F	N/A	N/A
Pen 3	Nonbreeding females	12	5357g *	7.2%

* indicates a significant difference between pen 3 when compared to pens 1 and 2.

The mineral composition of the diet offered and consumed by the bats was compared to the minimal mineral requirements reported for other mammals (Table 7-3). The minimum values for calcium and phosphorus followed the recommendation for fruit bats by the American Zoo and Aquarium Association Chiropteran Taxon Advisory Group (AZA Bat Tag) (Fascione 1995). All remaining mineral values used are based on

requirements for rats and were consistent with mammalian standards (Oftedal and Allen 1996). Both the diet offered and diet consumed in this study consistently equaled or exceeded the recommended requirements for all minerals examined. There was a difference among the minerals offered and the minerals consumed for the majority of minerals studied (Table 7-3). The amount of minerals offered is higher than that consumed, suggesting that bats consumed the lower nutrient foods from the diet and rejected the high nutrient foods.

Table 7-3. A comparison of the diet offered and consumed by *P. vampyrus* in this study to the recommendations of the American Zoo and Aquarium Association Chiropteran Taxon Advisory Group Bat and the mammalian standard.*

	Ca	P	Mg	K	Na	Fe	Cu	Zn	Mn	Ca : P
Mineral	%	%	%	%	%	ppm	ppm	ppm	ppm	ratio
requirement*	0.5	0.4	0.06	0.4	0.06	39	6	11	11	1:1-1:2
Diet Offered	0.9	0.7	0.1	0.9	0.2	93	8	49	25	1.3:1
Diet Consumed										
Pen 1	0.6	0.5	0.1	0.6	0.1	64	5	34	19	1.2:1
Pen 2	0.5	0.4	0.1	0.5	0.1	57	5	30	18	1.2:1
Control	0.6	0.5	0.1	0.5	0.1	70	6	36	19	1.2:1

*requirements for Ca and P are from Fascione 1995, all others are rat values from Allen and Oftedal 1996.
All values are averages for two days of collection in each pen, ppm = parts per million.

Because imbalances in the ratio of Ca to P leads to poor absorption and exacerbate deficiencies of both minerals, preferred dietary levels are typically expressed at a desired range of 1:1 – 2:1 for the Ca:P ratio (McDowell 1992). The diet offered and consumed by bats in this study met or exceeded the 1:1 Ca:P ratio.

The mineral composition of the diet offered to bats in pens 1 and 2 was identical (Table 7-4). The non-breeding females in Pen 3 received significantly less Ca, P, Mg, K, and Na than the lactating groups, presumably because the volume of diet offered in pen 3

was half of the volume offered in pens 1 and 2 and significantly lower ($p < 0.01$) for all macrominerals studied (Table 7-2). Rejected food, or orts, differed ($p< 0.01$) in mineral concentration for every mineral but Mn between the two pens containing lactating females. Given that they were offered an identical diet, this suggests that the females of pen 2 rejected more of the nutrient rich foods such as sweet potatoes, carrots and kale than the females in pen 1. The control group was significantly lower ($p < 0.01$) than pens 1 and 2 for all minerals studied. This reflects the fact that Pen 3 did not have any leftover food. This is a term called licked-bowl-clean in captivity (D. LeBlanc, pers.comm.). If it persists over several days, the diet volume offered is often increased. However, the control bats were overweight and did not need additional food, but overate as is often typical of non-breeding captive bats (Courts and Feistner 2000).

The mineral content of the feces of all three pens was homogeneous. Some minerals such as Ca are highly conserved in animals and appear only in minimal amounts in the feces when they are deficient in the diet (Ammerman 1995). Despite the ingestion of only half the minerals by the control bats in pen 3, their fecal matter contained similar amounts of minerals to the bats in pens 1 and 2. This suggests that the bats in pen 3 were not in mineral stress. Similarly, mineral retention was very similar for bats in pens 1 and 2 but were significantly different ($p < 0.1$) for those housed in pen 3. Mineral intake was presumably lower for bats in pen 3 because the diet volume placed in that pen was half that given to the other pens. Lactating females were significantly higher ($p < 0.1$) than the control females for mineral apparent absorption of most of the minerals examined in this study, excluding Cu and Na.

Table 7-4. A comparison of the mineral amounts for diet, orts, ejecta, mineral intake, mineral retention, and apparent absorption of minerals between two pens (1,2) of reproductive females *P. vampyrus* and one pen (3) of non-reproductive female *P.vampyrus*.

	Pen mg/g	Ca mg/g	P mg/g	Mg mg/g	Na mg/g	K mg/g	Cu mg/g	Mn mg/g	Fe mg/g	Zn mg/g
Diet Offered	1	1151	901	153	250	1107	1	3	12	6
	2	1276	1000	170	277	1227	1	3	13	7
	3	863*	676*	115*	187*	830*	1	2	9	5
Orts	1	137§	99	17§	29§	111§	0.1§	0.3	1§	1§
	2	313	223	36	66	230	0.2	0.3	3	2
	3	0*	0*	0*	0*	0*	0*	0*	0*	0*
Ejecta	1	229§	180	37	63	284	0.3	0.5	2	1
	2	194	174	33	56	285	0.2	0.3	2	1
	3	237*	370*	40	62	304	0.2	0.6	2	1
Feces	1	224	124	28	27	153	0.2	0.6	2	2
	2	250	147	27	22	119	0.2	0.8	3	3
	3	280	105	30	17	144	0.2	0.7	3	2
Mineral Intake	1	785	622	99	158	711	0.7	2	8	4
	2	769	602	100	155	713	0.7	3	8	4
	3	625*	490*	75	125*	526*	0.6	2	7	3
Mineral Retention	1	560	468	72	131	559	0.5	2	6	2
	2	518	438	73	133	594	0.5	2	5	2
	3	345*	372*	45*	108*	382*	0.4	1*	4	1*
Apparent Absorption %	1	71	72	72	83	79	72	74	69	44§
	2	68	66	73	85	83	74	71	63	38
	3	56*	49*	60*	87	72*	71	62*	55*	33*

§ indicates a significant ($p<0.01$) difference between pens 1 and 2

* indicates a significant ($p<0.01$) difference between pen 3 when compared to pens 1 and 2. Pens 1 and 2 contained lactating *P. vampyrus* females with pups, pen 3 contained non-breeding females and served as a control. Mineral values are averaged for both days of the trials and all values are reported in mg unless otherwise indicated.

Discussion

Both the diet offered and consumed by bats in this captive study was adequate to maintain other mammalian species in a non-breeding reproductive state and met the recommendations of the AZA Bat Tag for Ca and P (Fascione 1995, Allen and Oftedal 1996). The discrepancy between the offered nutrient levels and nutrients actually consumed suggests that bats chose less nutrient dense foods and rejected mineral rich

foods. Elevated apparent absorption values indicate that lactating females and their pups were mineral stressed for most of the minerals evaluated while consuming a standard diet that met nutrient recommendations for non-breeding bats.

Although the food fed to bats in this study met the recommendations for captive fruit bat diets, the mineral requirements for lactating females and rapidly growing young fruit bats remain unknown. Requirements for these groups are usually greater than for non-reproductive adults and are reflected in higher apparent absorption values (Kung et al. 1998). Apparent absorption is a measure of mineral retention expressed as a percentage of mineral intake. Higher absorption may indicate a greater physiological need (Keen et al. 1986, NRC 1989, Koo and Tsang 1991, Brink et al. 1992, Bronner et al. 1992). Animals that are deficient in a mineral (e.g., Ca) compensate by absorbing a maximum amount from the diet, which results in an elevated mineral absorption value (Ammerman 1995). For example, growing children may absorb up to 75% of ingested Ca as compared to 20-40% typically absorbed in adults (RDA 1989). In the present study, the pens that contained lactating females and their growing offspring had higher apparent absorption values for almost all minerals studied when compared to the control group of non-reproductive females. Elevated absorption levels indicate that lactating females and pups may be mineral stressed compared to the control group of non-breeding females. Minerals are most likely deficient in their diet because of the nutrient demands of prolonged lactation and rapid growth (Barclay 1995).

Calcium is of particular importance for lactating females and their rapidly growing pups (Kunz and Hood 2000). Young bats are not able to fly or forage independently until they have achieved nearly adult dimensions (Barclay 1995).

Consequently, near the end of lactation, female bats must feed young that are nearly adult size (Kunz and Stern 1995, Kunz and Hood 2000). Thus, it is important to provide adequate Ca in diets of lactating females. The discrepancy in Ca between the diet offered and consumed by bats suggests that *P. vampyrus* rejects many of the high Ca foods offered to them. Foods such as raw sweet potato, kale, and spinach are high in nutrients, but are fibrous and low in sugar, and are not normally encountered by wild bats (Oftedal and Allen 1996). These foods were consistently rejected by *P. vampyrus*. These results are consistent with the preferences of fruit bats from other studies, where bats preferred soft, non-acidic, low nutrient fruits with high sugar content (Parry-Jones and Augee 1991, Chapter 3, Courts and Feistner 2000). Therefore, high-Ca foods and Ca supplements should be in a form that is actually consumed by *P. vampyrus* or the Ca will be wasted.

The specific mix of produce and items fed to captive animals reflects ideas about food selection in the wild, food preferences demonstrated in captivity, established feeding practices, and local market availability of the food (Oftedal and Allen 1996). The diets prepared for fruit bats and other frugivores in captivity usually consist of chopped fruits and vegetables, manufactured primate pellets, and supplements such as vitamin E or Ca in an attempt to provide a balanced diet (Courts and Feistner 2000). However, captive diets also include commercially grown fruits and vegetables that are preferred by humans and are high in sugar (Oftedal and Allen 1996). Fruits such as apples and pears are used the most frequently and in the greatest quantities in captive diets because they are readily commercially available (Courts and Feistner 2000). Apples and pears together were almost half (43%) of the food that was fed to bats in this study. Commercial agricultural

fruits are generally depauperate in minerals and higher in sugar when compared to fruits available to flying foxes in the wild (Nelson et al. 2000a).

Based on the results of this study, the following changes in diet and procedures concerning captive pregnant or lactating female bats and their rapidly growing pups for *P. vampyrus* and other flying foxes are proposed.

1. Reduce the amount of apple in the diet and replace it with nutrient-rich, preferred fruits such as figs and papaya. Apples can comprise 36% of captive diets, but are nutrient poor and not preferred by bats (Oftedal and Allen 1996, Courts and Feistner 2000). Figs provide a very concentrated source of minerals and are a dietary staple of *P. vampyrus* in the wild (O'Brien et al. 2000, Nelson et al. 2000a, Stier and Mildenstein 2001). Papaya is a preferred fruit of wild flying foxes in American Samoa and it is rich in Ca (Chapter 3).

2. Increase mineral availability, particularly for Ca, in pens containing pregnant and/or lactating females and growing pups. Commercial mineral and Ca licks, Ca supplements, preferred tree leaves (Banack 1996, Chapter 4), and high Ca foods such as figs will increase mineral availability to supplement the standard diet given to all bats (O'Brien et al. 2000, Nelson et al. 2000b).

3. Do not breed *P. vampyrus* females every year. Because the Ca demands of raising several young in sequential years may result in osteoporosis (Studier et al. 1994a, Keeler and Studier 1992), females should have alternate years between births to rebuild their mineral reserves and the bone matrix donated to build the skeletons of their offspring (Bernard and Davison 1996).

4. Wean the offspring earlier from their mothers. This would reduce the occurrence of opportunistic and almost fully-grown offspring from continuing to suckle from their mothers, and would decrease both the volume of milk produced by the female and the duration of lactation (McDowell 1992). A shortening of the weaning period may reduce the amount of skeletal Ca donated by the female and allow her a longer recovery time to rebuild her Ca reserves following lactation (Bernard and Davison 1996, Keeler and Studier 1992).

Wildlife nutrition is still a very young science (Robbins 1993). Much research remains to be done, particularly on exotic animals such as fruit bats. Easy access to plentiful amounts of food in the captivity allow for selection of nutrient-poor, sugar-laden foods preferred by bats. Replacement of these fruits with preferred fruits that are also

high in mineral nutrients may reduce overeating and food waste. Lactating female Malayan flying foxes and their pups can survive and reproduce on the diet currently recommended and fed in captivity, but they are likely mineral stressed when fed this diet. Additional minerals are needed for pregnant, lactating, and rapidly growing animals. A greater volume of the standard diet given to each bat in a pen is not sufficient to meet their mineral needs. Instead, they require access to mineral rich sources such as figs, preferred tree leaves, commercial mineral licks and dietary supplements in addition to their standard diet (Chapter 3,4). Access to these rich mineral sources may reduce absorption rates and mineral stress in pregnant or lactating bats. If mineral stress in captivity can be resolved for lactating female *P. vampyrus*, one of the world's largest flying foxes and therefore the most likely to have the greatest mineral requirements, it is assumed that other captive bat species fed a similar diet while pregnant or lactating will not experience mineral stress. Future work should evaluate the effectiveness of various supplements and dietary changes and their associated effect on mineral absorption levels.

CHAPTER 8
SUGAR CONCENTRATION PREFERENCES OF TWO SPECIES OF BLOSSOM-BATS (*SYCONYCTERIS AUSTRALIS* AND *MACROGLOSSUS MINIMUS*) IN PAPUA NEW GUINEA

Introduction

Blossom bats are the smallest members of the family Pteropodidae of the Old World fruit bats. Both *Syconycteris australis*, the northern blossom bat, and *Macroglossus minimus*, the southern blossom bat, have large geographic distributions including Indonesia, Southeast Asia, Papua New Guinea and northeastern Australia (Figure 8-1). Blossom bats have remarkable plasticity in their temperature regulation and energetics. They inhabit areas with temperatures ranging from tropical to temperate in climate. Blossom bats live in a variety of habitats, including disturbed successional forests, primary lowland forest, and montane rain forest (Bonaccorso and McNab 1997). The distribution of *S. australis* extends farther south than any other small Australian megachiropteran and borders on areas with a temperate climate (Law 1994). *M. minimus* has a wide distribution but is limited to more tropical areas (Geiser et al. 1996).

Morphologically, the two species of bats are almost identical in size and mass (a.12-20 g). However, *S. australis* is distinguished from *M. minimus* by a more robust dentition that is better suited for feeding on fruits and figs (*Ficus* sp.), in addition to nectar and pollen of wild and domestic bananas (Flannery 1995a, Bonaccorso 1998, Winklemann et al. 2000). Although a dietary generalist in Papua New Guinea, *S. australis* is a nectar specialist in Australia, and feeds on blossoms of 18 rainforest species and from flowers of cultivated bananas (Law 1992, 1994, 2001). *M. minimus* is a nectar

specialist in Papua New Guinea, preferring the nectar of domesticated bananas, coconuts and mangroves (Gould 1978, Heideman and Heaney 1989, Bonaccorso 1998).

Specimens of *M. minimus* taken from New Guinea lacked fruit in the stomach (McKean 1983) and captive individuals refused to eat fruit when it was offered to them (Bonaccorso 1998).

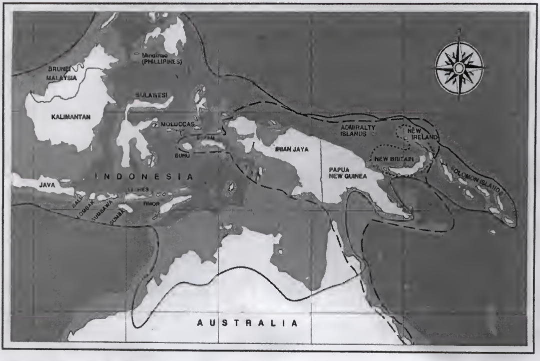

Figure 8-1. Distribution of blossom bats in Meganesia. Solid line is *Macroglossus minimus*, broken line is *Syconycteris australis*. From Bonaccorso and McNab 1997.

Blossom bats are highly suitable for studying preferences in nectar concentration. They are small, volant homeotherms with a high metabolic rate and no means of storing large energy reserves (Lemke 1984). Such species might be expected to respond markedly to subtle changes in resource availability and to choose high quality food areas (Law 1995). Several studies have suggested that blossom bat abundance may be limited by food availability and that bats are energy-sensitive foragers (Law 1992, 1993b, 1994).

Law (1993b) performed concentration preference tests on *S. australis* in Australia in which bats were given a choice of several concentrations of honey water. He found that blossom bats showed a distinct preference for the 40% solution (97% preference), to the exclusion of the less concentrated 10% and 20% solutions. When fed *ad libitum*, captive S. *australis* consumed 174% of its body mass in nectar each night. This study was conducted in Australia, where *S. australis'* habitat includes cold, wet winters and dry summers (Law 1994). Exposure to the low temperatures (only 6.7-7.7 °C) in the winter months requires a substantial amount of energy for thermoregulation, and can make food a limiting resource for *S. australis* (Law 1993a, Geiser et al.1996). Law (1994) calculated the metabolic costs for bats in the winter exceeded 4.5 x of their metabolic rate. Field metabolic rates of *S. australis* in Australia were the highest known for a mammal its size; more than seven times higher than their basal metabolic rate (Geiser and Coburn 1999). When supplemental food was made available, local populations of *S. australis* increased rapidly (Law 1995). Together, these studies suggest that energy may in fact be limiting to blossom bats in Australia.

Most studies of blossom bat metabolism and energetics have been performed on *S. australis* and *M. minimus* in Australia. Little is known about the energetic stress of these two species in Papua New Guinea. The subtropical and temperate climate and distinct seasonality in Australia may subject blossom bats to energy stressors not found in the more tropical and benign climate of Papua New Guinea. Therefore, my study attempted to examine three ideas. First, what nectar concentrations do blossom bats in Papua New Guinea prefer? If they are as energy stressed in Papua New Guinea as they are in Australia (Law 1993b), they should prefer the most concentrated nectar

concentrations available. Second, are there sugar preferrence differences between *S. australis*, a feeding generalist, and *M. minimus*, a nectar specialist in Papua New Guinea? It is predicted that *M. minimus*, a nectar specialist, would choose the higher concentrations because this species is completely dependent on nectar as a food source. Lastly, I attempted to identify if there were differences in energy requirements and consumption patterns between sexes or age groups of each species.

Methods

Nectar Concentrations

Sugar concentrations for the nectar preference trials were based on field measurements of banana nectar in Kau Wildlife Area on 1 July, 4 July, and 12 July 1999. Kau Wildlife Area is adjacent to Baitabag Village in Madang Province, Papua New Guinea. Kau Wildlife Area is an 800 ha reserve of lowland forest that contains primary old growth, successional, and riparian forest (Winklemann et al. 2000). Flowers of 20 banana plants were monitored in three all-night sessions that lasted from 1900-0600 h. Blossom bats were often seen or heard during nectar sampling periods. Nectar was collected non-destructively with a blunt-edged syringe and samples were placed on a hand-held sugar refractometer (Model # 300010, Sper Scientific, Scottsdale, AZ) to determine sucrose content on a g solute per 100g-solution basis (Bolten et al. 1979). Sugar concentration ranged from 5-24% sucrose equivalents with a mean of 13.24 $\pm$ 3.75% (SD) (n= 151) but nectar concentrations varied widely during the night (Figure 8-2). The nectar concentration values found here are consistent with those found in other bat pollinated flowers (Helverson and Reyer 1984, Kress 1985, Itino et al. 1991). From these results, three concentrations were selected for the nectar concentration preference

experiments, 7%, 15% and 30% nectar, because those values are typical of those available to blossom bats in their native habitat.

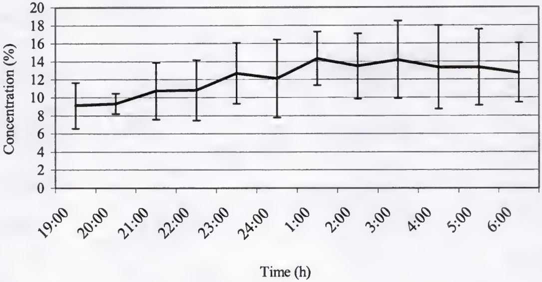

Figure 8-2. Banana flower nectar concentrations. Concentration values have been averaged for three nights.

Preference Tests

Twenty blossom bats were used to determine nectar concentration preferences; 15 *S. australis papuanus* (7 females, 8 males) and 5 *M. minimus nanus* (2 females, 3 males) (Table 8-1). Bats were captured in mist nets at Kau Wildlife Area and transported to the Christensen Research Center in cloth bags. Sex, body mass (g) and forearm length (mm) were recorded for each bat. Age was determined as adult or juvenile based on body mass, forearm length, and fusion of the metacarpal epiphyses (Anthony 1988). Bats were placed individually in 1.5 x 1 x 1 m flight cages for four days. Sugar solutions (henceforth "nectar") were made by diluting honey with water in a 100 ml graduated cylinder. Concentrations were checked to within 1% by a hand-held refractometer (Atago NI #791, Vee Gee Scientific, Inc., Kirkland, WA).

In preference tests, I compared preference for the 15% to the 7% and 30% sugar solutions. Two separate trials were run on each bat. The first two days bats were given a choice of either 15% or 30% solution, and the second two days a choice between 15% and 7% solution. Thirty milliliters of each nectar concentration in a plastic dish were placed in each cage in late afternoon and then collected the following morning. The position of the feeders was switched daily to eliminate any preference for the left or right side. The amount of sugar solution consumed by the bat was determined by subtracting the volume remaining the next morning from the original 30 ml offered. A predetermined evaporation constant of 2 ml was subtracted from all consumption volumes. Bats were released in the morning if they ate less than 10 ml the previous night. Results from two days of each trial were averaged to calculate a mean preference value for each trial for each bat. A preference index was calculated as the ratio of the consumption of sugar A divided by the total consumption (A+B) (Martinez del Rio 1990, Law 1993b).

A repeated measures test was used to analyze nectar consumption patterns (SAS 6.12, SAS 1999), because the four consecutive nights of preference tests were not indepe

ndent measurements. Consumption levels were adjusted for the body mass of the bat as a continuous covariate while I tested for interactions between gender, species, and reproductive status. There was no significant difference ($p = 0.65$) between *S. australis* and *M. minimus* for consumption volumes; thus they were grouped together for subsequent analysis. Because there was a significant interaction between gender and age ($p = 0.002$) that affected consumption, I performed pairwise comparisons using Tukey-Kramer's multiple comparisons test to determine which pairwise comparisons were different among those factors (Sokal and Rohlf 1995).

Table 8-1. Bats used in the nectar preference tests. Daily average and total consumption of all concentrations combined are based on four nights of experiments.

Species	Gender	Group	Body mass (g)	Length of forearm (mm)	Average consumption (mL)	Total consumption (mL)
S.australis	Female	Adult	19	44	23	92
S.australis	Female	Adult	18	43	25	100
S.australis	Female	Adult	20	45	29	115
S.australis	Female	Subadult	17	42	28	110
S.australis	Female	Subadult	16	45	24	95
S.australis	Female	Subadult	17	43	20	81
S.australis	Female	Subadult	20	41	21	85
S.australis	Male	Adult	17	45	18	73
S.australis	Male	Adult	19	46	17	69
S.australis	Male	Adult	20	42	15	59
S.australis	Male	Adult	20	43	24	97
S.australis	Male	Adult	20	46	22	88
S.australis	Male	Adult	18	48	18	72
S.australis	Male	Subadult	18	38	23	92
S.australis	Male	Subadult	15	38	28	113
M.minimus	Female	Adult	17	44	27	106
M.minimus	Female	Subadult	18	42	22	89
M.minimus	Male	Adult	18	43	18	72
M.minimus	Male	Adult	20	44	21	84
M.minimus	Male	Adult	23	42	26	103

Results

A total of 90 test-days were completed on 20 wild-caught blossom bats. Both *S. australis* and *M. minimus* preferred the 15% sugar solution to the 30% solution ($p < 0.001$) choosing the 15% solution 54% of the time and the 30% solution 46% of the time (Figure 8-3). When presented with the choice between the 15% and 7% solutions, both species of bats preferred the 15% solution (69% preference) to the 7% solution (31% preference, ($p < 0.01$). (Figure 8-4) Overall, the 15% solution was preferred to either the 7% or 30% nectar solution. Together, both species consumed an average of 21.59 ± 6.62

ml (SD) of nectar per night. Average consumption for all bats was 117.86 ± 39.48% (SD) of their body mass per night

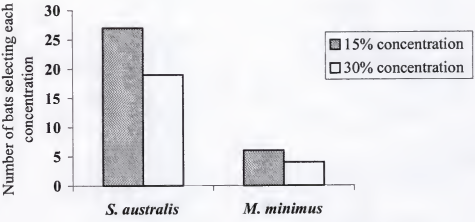

Figure 8-3. Results of 15% or 30% nectar-preference test.

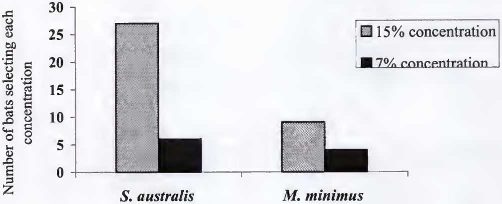

Figure 8-4. Results of 15% or 7% nectar-preference test.

Females consumed more nectar solution ($p = 0.04$) than males (Figure 8-5). Adult females consumed an average of 103.25 ± 9.71 mls (SD) per bat over four nights, and males consumed an average of 79.67 +14.30 mls (SD). Females consumed 160.38 ± 26.57% of their body mass in nectar every night. Males consumed only 108.86 ± 40.03% of their body mass.

As a group subadult females consumed an average of 92.00 ± 11.31 mls (SD) per bat over four nights, and subadult males consumed an average of 103.25 ± 9.71 mls (SD). The largest volume of nectar consumed in a single night was by a subadult male *S. australis*, who consumed 253% of his body mass in nectar (38 ml).

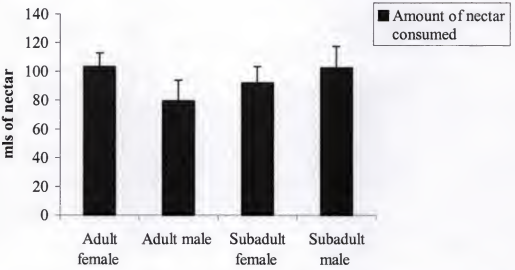

Figure 8-5. Average amount of nectar consumed over four nights for each age class.

Discussion

This study found that *S. australis* and *M. minimus* prefer the same nectar concentrations (15% sucrose) in preference tests, and both species consume them in similar volumes. Adult females consumed more than males in both species, and subadults consumed more than adults. Adult males consumed the least nectar volume of all groups tested. A lack of preference for the highest concentration of 30% nectar indicates that blossom bats in Papua New Guinea are not as energy stressed as they are in Australia, and that they are not energy-sensitive foragers (Law 1993b) in the more benign climate of Papua New Guinea.

Blossom bats actively seek food sources with a specific nectar content (Law 1993b, Baker et al. 1998). Bats typically chose the highest concentration of nectar available to them to maximize their energy return (Law 1993b, Roces et al. 1993, Law 1994). However, in Papua New Guinea, the climate is much warmer and constant, and bats from lowland Papua New Guinea may not be as energy or food stressed as they are in Australia. Ambient temperatures in Papua New Guinea are 25-30°C almost all year, and there is equitable rainfall throughout the year (Bonaccorso and McNab 1997). Nearby forest and garden habitats provide continuous fruits and flowers during both the wet and dry season (Winklemann et al. 2000). In Papua New Guinea, *S. austalis* has the smallest home range of any bat yet studied. This is probably due to the relatively stable and abundant food resources, generalized diet of this species, and high availability of roost sites. (Winklemann et al. 2000). Home ranges for *S. australis* in Papua New Guinea were 2.7-13.6 ha (Winklemann et al. 2000). Conversely, home ranges were more than 100 fold larger (12 to 1796 ha) in Australia due to dispersed and less stable food resources (Law and Lean 1999). Papua New Guinea and Australia present very different energetic stresses and resources to blossom bats living in these two environments.

Climate can affect the metabolic rates of blossom bats. Populations of *S. australis* and *M. minimus* from Papua New Guinea responded to progressively cooler temperatures with elevated rates of metabolism. *S. australis* found in the warmer lowland forests of Papua New Guinea has a mean basal rate of metabolism 67% of that expected for its body mass. In contrast, *S. australis* from the cooler New Guinea highlands had a mean basal rate of metabolism 110% of the expected value for a mammal of its body size (Bonaccorso and McNab 1997). Similarly, the metabolism of *M. minimus* in its tropical

range is only 57% of that expected, and is the lowest relative rate of metabolism known for a nectar-feeding bat. *Macroglossus minimus* from the colder highlands had a basal rate that was elevated to 94% of the expected value for its body mass (Bonaccorso and McNab 1997, McNab and Bonaccorso 2001). These studies suggest a lack of energy stress in the warmer climate and higher metabolic requirements as a result of cooler temperatures (McNab and Bonaccorso 2001). Average nectar consumption for all bats in this study was 118% of their body mass per night, whereas in Australia bats consumed 174% of their body mass in nectar nightly (Law 1993b).

Shallow torpor can be an energy saving mechanism for bats living in the tropics (Bartels et al. 1998, Coburn and Geiser 1998). *Syconycteris australis* entered shallow torpor daily to save energy when food and water were withheld (Coburn and Geiser 1998). The fact that *M. minimus* has a less well-developed pattern of torpor than *S. australis* may indicate that this species has not had to adapt to the level of energetic stress that the more widespread *S. australis* has adapted to (Bartels et al. 1998). More pronounced torpor in subtropical *S. australis* may be due to low or unpredictable nectar availability, or short nights that limit the time available for foraging, and extended time at the day roost (Coburn and Geiser 1998). The energy-saving benefits of torpor may not be used often with the tropical climate and year round food resources of Papua New Guinea (Bonacccorso and McNab 1997). Overall, heterothermic bats seem to be more influenced by their size and the climate of their habitat than by their diet or suborder membership (Geisner et al. 1996).

A preference for the 15% solution rather than the 30% solution may not be as counterintuitive as first expected. It may actually be more profitable to consume nectars

of lower concentration to elude predation. There is a point beyond which an increase in sugar concentration actually decreases the rate of energy yield to the nectar feeder (Heyneman 1983). Lower sugar concentrations allow a more rapid fluid uptake, but a lower award per unit volume of nectar. Higher nectar concentrations offer a much more energy rich reward but limit the uptake of nectar because the rapid increase in nectar viscosity sharply reduces the rate of fluid intake (Heyneman 1983, Roces et al. 1993). Ambient temperatures influence nectar flow rates. Energy return is higher for a 10% sucrose solution at 30°C than for a 22% concentration at 15°C (Heyneman 1983). Hence, in Papua New Guinea, where ambient temperatures range from 28-30°C, it is actually advantageous for nectar feeding bats to feed on lower concentrations due to the increased flow rates at these temperatures. This decreases the time spent at a flower and may result in lower predation levels. The preference of *S. australis* for 15% nectar concentrations makes sense in the context of the warmer temperatures of its New Guinean habitat. Ambient temperatures place little metabolic stress on the bats, and are near optimum for nectar flow. Other bat species consuming nectars with a concentration of 18-21% had a balanced daily energy budget (Helverson and Reyer 1984).

In this study adult females consumed a greater volume of liquid than males in both populations, and subadults of both species consumed more than adults. Overall, blossom bats in this study consumed 118% of their body mass, but females consumed an average of 160% of their body mass daily. Sixty-seven percent of the highest nightly consumption volumes (>30 ml) were consumed by females, and they chose the 30% solution more often than did males. These results are potentially the result of elevated metabolic rates due to the increased energy demands of pregnancy and lactation. For *M.*

minimus in Papua New Guinea, breeding is asynchronous and young are born throughout the entire year. At any specific locality it is possible to collect females that are nursing young, pregnant, or with an early implanted blastocyst or near-term fetus, or non-pregnant with stored spermatozoa (Hood and Smith 1989). Adult female *S. australis* may give birth to two young per year (Bonaccorso 1998) at high energetic cost. The energy requirements and food intake of pregnant females can be up to 32% higher than non-reproducing females (Mattingly and McClure 1982, Thompson 1991).

Lactation incurs a high energetic cost, being twice as energetically expensive as gestation (Racey and Speakman 1987). Barclay (1989) found that as lactation progressed, female bats spent more time, and began foraging earlier, to compensate for the energy demands of lactation. Post-lactational females may have been over-consuming to reduce the energetic effects of recent lactation. Other studies have also found that populations of breeding females ate significantly more than mature males of the species (Clark 1980).

Subadult blossom bats consumed more nectar than adults of both species. Similar to females, this may indicate a response to a temporary energetic deficit. Subadults conceivably consume more nectar than adults because they are rapidly growing, and their need for increased energy has resulted in elevated metabolic demands. Data on post-natal growth rates of free-ranging tropical frugivores do not exist (Kunz and Stern 1995, but see Kunz and Hood 2001), but as a general rule, small mammals have high growth rates. The largest volume of nectar consumed in a single night was by a young male *S. australis*, who consumed 253% of his body mass in one evening. Subadult bats may be responding to temporary but intense energetic stress during periods of high growth. Adult male fruit bats were overall more stationary, and only moved half as far as females or

subadults in their nightly foraging flights (Heideman and Heaney 1989). This may indicate that adult males are not energetically stressed, as are females and subadults.

In conclusion, both species of blossom bats preferred 15% nectar solution to either 30% or 7% nectar. This may indicate that they are not as energy stressed as blossom bats that occur in more temperate and seasonal environments in Australia. The second most preferred nectar concentration was the 30% solution, which is energy rich and may have been used for bats that were temporarily physiologically stressed, such as parous females or growing subadult bats. The 7% solution may offer too little energy and not be profitable for bats to consume. Preference for the 15% solution may also be related to the nectar flow rates in the warm temperatures of New Guinea. The high temperatures and constant food availability of Papua New Guinea seems to result in limited metabolic stress to blossom bats that live there. Reproductive females and subadults may be more energy stressed than males, but on a temporary basis.

CHAPTER 9
CONCLUSION AND CONSERVATION RECOMMENDATIONS

Testing the Calcium-Constraint Hypothesis

Robert Barclay (1994,1995) first proposed in his calcium-constraint hypothesis that calcium rather than energy may limit reproduction in female bats. Reproductive female bats are often calcium deficient following pregnancy and lactation (Kwiecinski et al. 1987, Keeler and Studier 1992, Studier et al. 1994a, Studier and Kunz 1995, Bernard and Davison 1996). This research experimentally tested the calcium-constraint hypothesis using three different species of fruit bats in four different geographic areas. I predicted that fruit bats, particularly reproductive females, would seek out and use calcium sources to supplement their diet and to relieve calcium deficiencies.

The most extreme test of the calcium constraint hypothesis included captive lactating Malayan flying foxes (*P. vampyrus*), one of the world's largest flying foxes (Chapter 7) with the assumption that their calcium requirements would be very high. Malayan flying foxes did not select high calcium foods in this study. Instead, they rejected high-calcium foods and preferred fruits high in sugar. *P. vampyrus* did not appear to meet its calcium needs and showed elevated absorption levels, a potential sign of calcium deficiency.

Tongan fruit bats (*P. tonganus*) in American Samoa also rejected calcium-rich fruits and instead preferred high-energy, sugar-rich fruits in fruit choice experiments (Chapter 3). They also preferred agricultural areas while foraging throughout the island landscape (Chapter 6). Mineral metabolism experiments indicate that wild Tongan fruit

bats did not meet many of their mineral requirements while consuming a diet consisting primarily of agricultural fruits (Chapter 5). *P. tonganus* consumed only 1/8 of the recommended calcium requirement on their chosen diet. In addition, apparent absorption values for the Tongan fruit bat population were higher for lactating female *P. vampyrus*, potentially indicating mineral deficiency throughout the Tongan fruit bat population.

Both Malayan and Tongan fruit bats did not seem to be selecting foods in a manner that maximized their calcium intake. Their preference for fruits high in sugar may instead indicate an energy constraint. To examine bat consumption to maximize energy, I utilized two of the smallest members of Old World fruit bats, the blossom bats *Syconycteris australis* and *Macroglossus minimus* (Law 1992, 1993b, 1994). Neither species preferred the most concentrated energy source and instead preferred the intermediate 15% sugar solution. However, females and young adults consumed the largest volumes of nectar, possibly indicating energy stress among those groups due to reproduction and rapid growth.

Mineral Compensation

Tongan fruit bats did seek out and consume calcium-rich resources on a limited basis. The strongest indicator that *P. tonganus* may be compensating for their calcium-poor diet was the frequency of leaf-consumption by captive bats (Chapter 4). Most of the Tongan fruit bats in captivity (83%) ate leaves, which had the potential to increase their calcium consumption by 46%. Although not high in sugar or energy nor especially palatable, leaves represent a readily available and consumed calcium-rich source of minerals (Kunz and Ingalls 1994, Kunz and Diaz 1995, Nelson et al. 2000b).

The second indicator that fruit bats may supplement their diet with additional calcium was seen in female use of calcium blocks (Chapter 3). Three times as many

females as males used the calcium blocks in the feeding experiments, with 100% use and the highest frequency of use among reproductive females, although the sample size was very small. The members of the group that were most vulnerable to calcium deficiency, including reproductive females and rapidly growing young males, used the calcium blocks most often. This may indicate that calcium is consumed to relieve deficiencies among these individuals.

Fruit bats may also compensate for a lack of calcium in their diet by their unique manner of feeding, which possibly results in greater calcium availability (Chapter 5). By consuming mostly fruit juices, minerals like calcium may be swallowed in solution rather than bound to fiber, and may result in a higher degree of bioavailability (Ammerman 1995). A specialized gastrointestinal tract may also help to increase calcium absorption.

Tongan and Malayan Fruit Bat Dietary Choice

Tongan and Malayan females bats did not appear to consume a nutritionally adequate diet. Their diet was marginal for most of the macronutrients examined in this study and especially poor in calcium. If and how Tongan and Malayan females are able to rebuild their skeletons following pregnancy and lactation, and support continued population growth remains unknown. Because female reproduction issues are critical to the health of the population and the continuation of the species through time, the food choices made by both *P. tonganus* and *P. vampyrus* seem maladaptive.

The fruits chosen by these two species, and the mineral intake that results, may not be typical of other fruit bat species. The Samoan fruit bat, *P. samoensis*, co-occurs with *P. tonganus* in American Samoa, but feeds primarily in native forest, consuming a wide variety of calcium-rich native fruits including figs (Trail 1994, Banack 1996). Similarly, *A. jubatus*, which co-roosts with *P. vampyrus*, consumes a diet rich in figs and

native fruits (Stier and Mildenstein 2001). Most fruit bat species consume fruits from the native rainforest (Palmer and Woinarski 1999, Palmer et al. 2000, Brooke 2001) or even preferentially consume calcium-rich foods (Barclay 2002). Therefore, the results of this research may be typical of fruit bat species that use agricultural areas, but may be atypical of bats that utilize native forest for the majority of their feeding. It is unknown what Tongan fruit bats ate before the arrival of Polynesians 3,000 years ago and the planting of agricultural fruits (Kirch and Hunt 1997). Tongan fruit bats may have previously foraged in a manner more consistent with that of Samoan fruit bats and consumed a higher quality diet.

Summary

This research attempted to distinguish if reproduction in females was constrained more by energy or calcium (Barclay 1994, 1995). The results of this research indicate that bats forage to maximize their energy intake by preferring sugar-rich fruits. However, in times of physiological stress such as pregnancy and lactation, or during rapid growth by young bats, fruit bats may exhibit compensatory foraging for deficient minerals such as calcium. Use of leaves and calcium blocks may highlight a drive to relieve mineral deficiencies by consuming concentrated mineral sources.

Foraging habits of reproductive females were of special consideration in this research. However, generalizations derived from their choices are limited because of the small sample size of captured reproductive females. Much work remains to be done in the area of nutritional ecology to further distinguish what motivates food choice among Old World flying foxes and how reproductive females meet their nutritional requirements.

Conservation Recommendations

Major Threats to Fruit Bats

Human activities constitute the greatest threat to fruit bat populations. Fruit bats can be harmed either directly by hunting at the local or commercial level, or indirectly by large-scale destruction of habitat and roost disturbance. Fruit bats have long been consumed by local people (Wodzicki and Felton 1980, Cheke and Dahl 1981, Heaney and Heideman 1987, Rainey 1990). Hunting continues at both the local and commercial level, often as part of a luxury food trade (Racey 1979, Wiles and Payne 1986, Fujita and Tuttle 1991) or by commercial fruit growing operations, where fruit bats are considered pests (Loebel and Sanewski 1987).

Habitat loss and forest destruction are now considered the major factors contributing to fruit bat population decline (Cheke and Dahl 1981, Fujita and Tuttle 1991, Mickleburgh et al. 1992, Pierson and Rainey 1992). Loss of forest results in loss of both critical food and roosting resources as native forest is logged to make way for residential and/or commercial development and agriculture (Mickleburgh et al. 1992). Many fruit bat species roost deep in native forest, and disturbance to the roost, either accidental or intentional, can result in abandonment that can have population level effects during the maternity season (Wiles 1987b, Brooke 1998). Natural catastrophes such as periodic and destructive hurricanes can also lead to dramatic population declines in fruit bat populations (Craig et al. 1994b).

The National Park of American Samoa

The National Park of American Samoa on Tutuila, Ofu, and Olosega, American Samoa, is important regionally as a refuge for Tongan and Samoan fruit bats that reside there (Brooke 1998). The National Park provides primary forest and roosting locations

that are secluded from human disturbance and protected from hunting. Native forests are important to Samoan fruit bats for foraging and roosting (Banack 1996, Brooke 2001). Tongan fruit bats also favor isolated and inaccessible roosts in native rainforest (Brooke 1998). There are at least four traditional Tongan bat roost sites within the National Park on Tutuila that are currently in use (Brooke 1998) Additional roosts may be established within the National Park as native forest areas continue to dwindle as a result of the rapid human population growth (Brooke 1998, Craig et al. 2000). Although a substantial amount of forest is contained within the National Park, it may not be adequate to maintain viable populations of bats if forest clearance continues at the current rate (Rainey 1998). Any opportunity to enlarge the current boundaries of the National Park, particularly along the north coast of Tutuila, should be seized (Brooke 1998).

On a larger scale, long-term and landscape-level conservation plans must be enacted, and native forests set aside as conservation areas to protect current and future populations of fruit bats within oceanic islands in the South Pacific. A collection of conservation areas can act as refuges for bat populations and can serve to repopulate areas following hurricanes and other catastrophes.

Reducing Hunting Pressure and Bat Education Programs

A ten-year hunting ban, in effect since 1992, has reduced hunting in American Samoa (Craig and Syron 1992, Brooke 2001), but hunting of fruit bats continues (S. Nelson, pers. obs.). The hunting ban was enacted to address declines in fruit bat populations resulting from the combination of hurricane damage to the forest following three successive hurricanes, and the opportunistic hunting of bats immediately afterwards. Together, these factors contributed to an 80-90% decline in populations of

fruit bats (Craig et al. 1994b). When questioned, American Samoans were either largely uninformed of the ban, or killed and consumed bats despite it. Therefore, I suggest aggressive education programs in schools and villages explaining the reason for the hunting ban. I also suggest using the widely read local newspaper for articles explaining the ban, and to publish reminders of the hunting ban weekly or monthly. My experience talking to schoolchildren indicated that residents of the most remote villages on Tutuila were least aware of the role of bats in forest ecosystems and also engaged in the most bat hunting. Hence, these areas should be a priority for education programs on the island. Although discussed for Tutuila, American Samoa, these recommendations can be widely applied to other Pacific island ecosystems where bats are hunted unsustainably.

The fate of the native forest and the fruit bat populations that reside within them are interconnected. Native forest provides fruits and roosts to fruit bats, and fruit bats are crucial to the maintenance and integrity of native forests. Due to the paucity of alternative vertebrate pollinators and seed dispersers on isolated oceanic islands, fruit bat species are often considered keystone species (Cox et al. 1991, Rainey et al. 1995, Banack 1998). Thus, declines or extinctions among fruit bat populations may have long-term impacts on forest regeneration (Bonaccorso and Humphrey 1978, Thomas 1982, Rainey et al. 1995) and cascade effects on other species that reside in native forest (Rainey 1998). A loss or significant decline in bat populations may ultimately affect community structure, biodiversity and ecosystem function in simple systems such as oceanic islands.

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BIOGRAPHICAL SKETCH

Suzanne Nelson was born on April 12, 1972. She was raised in Mt. Prospect, Illinois, a suburb northwest of Chicago. She was the middle of three children. As a child she loved to be outside and spent most of her vacations at her grandparent's house in south Florida and at her aunt and uncle's farm in southern Illinois. After graduating from Prospect High School in 1990, she attended the University of Illinois at Urbana-Champaign and graduated in 1994 with a B.S. degree in Ecology, Ethology, and Evolution. She traveled to American Samoa for the first time as a volunteer wildlife biologist while taking a year off to travel after college. In 1995 she returned to the University of Illinois to study nutritional ecology of fruit bats in American Samoa for her M.S. degree with Dr. Edward Heske. She graduated with an M.S. in Biology in 1997. She taught at the University of Alaska-Anchorage before beginning her Ph.D. program at the University of Florida at Gainesville in 1998. While at the University of Florida, she was supported by the Luis F. Bacardi Graduate Fellowship as a student of Dr Steve Humphrey and Dr. Tom Kunz. Suzanne lives with her husband Darrin Masters and their golden retriever, Louie.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Stephen R. Humphrey, Chair
Professor of Wildlife Ecology
and Conservation

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2003

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